Chemical Library

OCT 2 1942

SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

CONTENTS OF THIS ISSUE

Growth Effects of Pyridine, Piperidine, Atropine Sulfate and Thiamin Chloride on Stramonium Seedlings 257
H. W. Youngken, Jr., and E. B. Fischer

A Note on the Healing Action of Allantoin, Allantoin Dipiperazine and Urea (Preliminary Report) 263
Frederick R. Greenbaum

A Study of Syrup of Hydriodic Acid 265
Howard Hopkins and C. O. Lee

A Study of Isotonic Solutions 270
William J. Husa and Oscar A. Rossi

Organic Mercurials: The Influence of Structure on Absorption and Excretion . . . 278
William H. Hunt, Lewis A. Walter and Russel J. Fosbinder

Bacteriostatic Properties of Certain Derivatives of Thiophene 281
H. W. Rhodehamel, Jr., and E. F. Degering

A Study of Athlete's Foot and Its Control, II . 284
R. E. Williamson and H. G. DeKay

Book Reviews 286

Abstracts 257–288

Advertising—Cover Pages 2, 3, 4, and pp. I to VIII

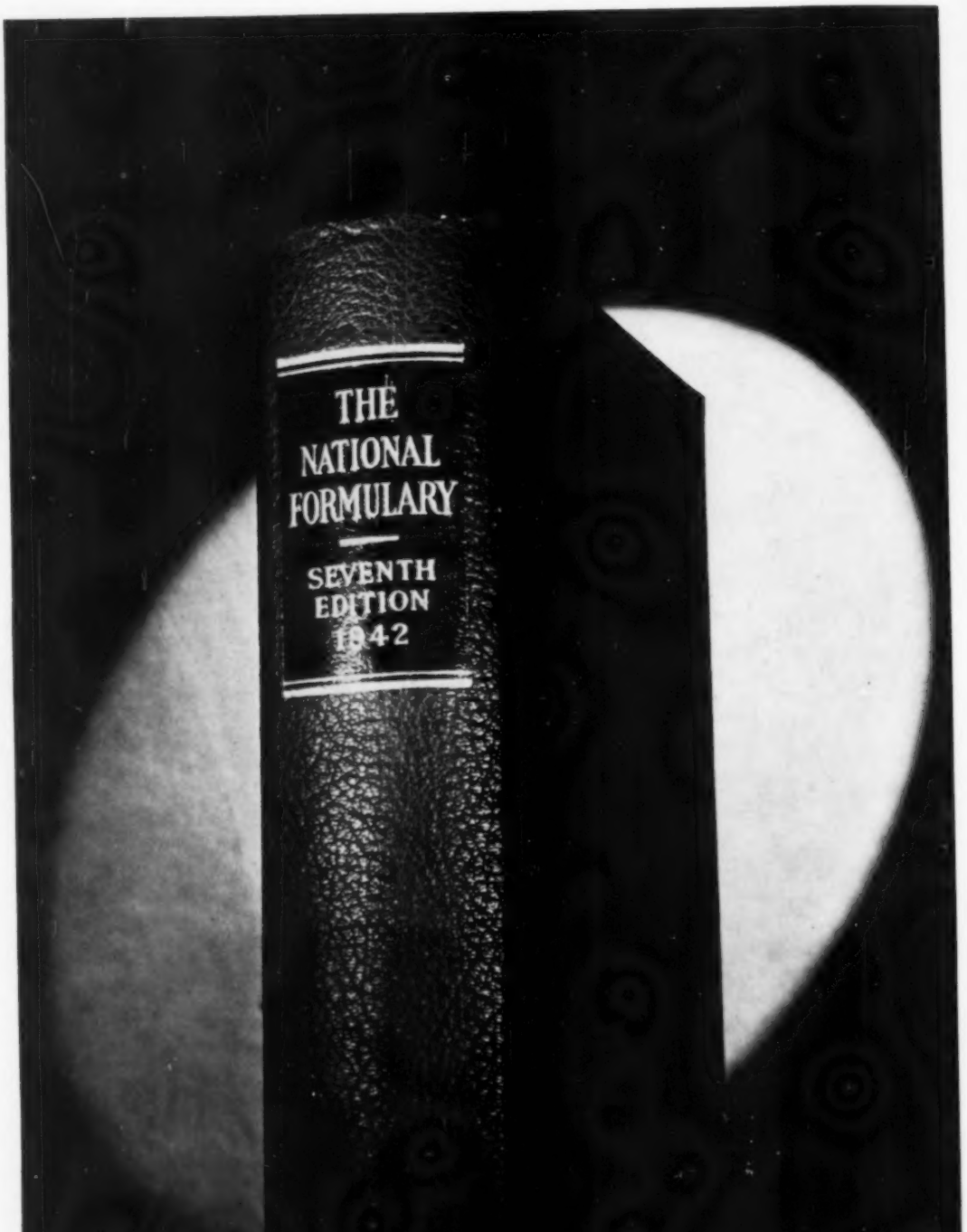
Published by the American Pharmaceutical Association. Publication office: 20th and Northampton Sts., Easton, Pa. Editorial office: 2215 Constitution Ave., Washington, D. C.

Annual Subscription: Journal of the American Pharmaceutical Association, complete $5.00; Canada, $6.00; Other Foreign, $6.50. (*a*) Scientific Edition, $4.00; Canada, $4.35; Other Foreign, $4.50; Single Numbers, $0.35; Canada, $0.40; Other Foreign, $0.50. (*b*) Practical Pharmacy Edition, $1.50; Canada, $1.70; Other Foreign, $2.00; Single Numbers, $0.15; Canada, $0.20; Other Foreign, $0.25.

No claims will be allowed for Journals lost in the mails due to: Failure to notify of change of address (thirty days' notice required); after a lapse of three months; failure to receive wrappers from returned Journals.

Entered as second-class matter January 23, 1917, at the Post Office at Easton, Pa., under the act of March 3, 1879, as 24 times a year: Scientific Edition monthly on the fifth. Practical Pharmacy Edition monthly on the 20th. Acceptance for mailing at a special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 10, 1918.

Vol. XXXI, No. 9 **SEPTEMBER, 1942**


THE
NATIONAL
FORMULARY
SEVENTH
EDITION
1942

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXI | *SEPTEMBER, 1942* | NUMBER 9 CONSECUTIVE NO. 17

Growth Effects of Pyridine, Piperidine, Atropine Sulfate and Thiamin Chloride on Stramonium Seedlings*

By H. W. Youngken, Jr., and E. B. Fischer†

Recent investigations on the treatment of plants with chemical substances have resulted in well-founded evidence that plants will respond to chemical feedings in varying ways. So numerous are the claims made that only a few, those pertaining to treatments of pharmaceutically important drug plants, can be cited here. Blakeslee, *et al.* (1), have shown that the alkaloid colchicine will produce marked effects on the chromosome arrangement and inhibitory effects on the growth habit of *Datura Stramonium* L. seeds and seedlings. Avery, *et al.* (2), have induced decided growth responses in *Nicotiana* species with the use of several auxins. Zopf (3) showed that indoleacetic acid, α-naphthaleneacetic acid, thiamin chloride, and ascorbic acid in varying combinations inhibited or accelerated growth responses in species of *Belladonna* and *Ricinus;* further, that simultaneous treatments with vitamin B_1 and vitamin C produced antidotal properties toward toxic doses of α-naphthaleneacetic acid.

On the basis of these and other findings (4) that indicate plants absorb chemicals in solution and are thus affected in growth, an experiment was planned to study the effects of pyridine, piperidine, atropine sulfate and thiamin chloride upon *Datura Stramonium* L. This experiment was to serve a twofold purpose: (*a*) to determine to what extent certain concentrations of these chemicals when applied to growing stramonium seedlings would produce toxic effects, and (*b*) to determine what effect, if any, the feeding of chemical substances with structures similar to the atropine nucleus might have upon the yield of this alkaloid in the leaves of mature stramonium plants.

The procedure of the first part of this plan has been completed and is presented with its results.

EXPERIMENTAL

The chemical substances used in the experiment consisted of chemically pure pyridine, piperidine, atropine sulfate and thiamine chloride. The relative strength of each follows in the text. Soil cultures and pure quartz sand cultures were employed for growing media. Soil cultures consisted of a fine grade peat soil obtained from the University of Minnesota Medicinal Plant Gardens. This was thoroughly sifted and mixed. Several nutrient solutions for use in quartz sand cultures were tried during the preliminary investigations. These included Knop's modified solution and two nutrient solutions from Hoagland and Arnon (5). One

* Presented to the Plant Science Seminar, August, 1941, meeting.

† Department of Pharmacognosy, College of Pharmacy, University of Minnesota, Minneapolis, Minn.

modified formula devised from the latter was found best suited for use. This nutrient formula is given in the following tables.

TABLE I.—NUTRIENT FORMULA

	Concentration, Cc./L.
Ammonium acid phosphate, 1 *M*	1
Calcium nitrate, 1 *M*	4
Potassium nitrate, 1 *M*	6
Magnesium sulfate, 1 *M*	2

To this solution a supplementary solution supplying boron, manganese, zinc, copper and molybdenum was added (Table II).

TABLE II.—SUPPLEMENTARY SOLUTION

	Concentration, Gm./L.
Boric acid	2.86
Manganese chloride	1.81
Zinc sulfate	0.22
Copper sulfate	0.08
Molybdic acid	0.02

One cubic centimeter of this solution (Table II) was added for each liter of nutrient solution at frequent intervals. This resulted in the concentrations shown in Table III.

TABLE III.—APPROXIMATE CONCENTRATIONS

	Parts per Million of Nutrient Solution
Boron	0.5
Manganese	0.5
Zinc	0.05
Copper	0.02
Molybdenum	0.01

In order to supply necessary iron a 0.5% iron tartrate solution was added about once a week to the nutrient cultures. Additions of this depended upon the chlorotic appearance of the plants. The *p*H of the solution was adjusted to approximately *p*H 6.

Quartz sand used in the experiment was supplied by the Minnesota Quartz Company, Mendota, Minnesota, and was assayed by the University of Minnesota Geology Department. It consisted of 99.6% of SiO_2 and 0.4% of limonite, calcite and iron. This was thoroughly washed before using.

Growing seedlings approximately 2 and 3 cm. in height, without the appearance of the young leaves but with two cotyledonary leaves, were transplanted from soil to each of a series of soil- and quartz sand-containing jars, and treated under glass. Two-gallon glazed earthenware jars were used. Each was equipped with suitable drainage systems.

The experiment[1] was divided into two parts: Part I consisted of the treatment of seedlings with five different concentrations of each of four chemical substances. The purpose of these treatments was to determine the minimum toxic concentration, or that concentration at which plants would show definite toxic effects when regularly saturated with the chemicals. A period of twenty days was allotted for these treatments. Part II consisted of treating quartz sand and soil cultures with a single concentration of each of the four chemical substances. This concentration was chosen only after the results of Part I could be ascertained and was based on these findings. Saturations were made once every three days for the soil cultures and twice weekly for the sand cultures of Part II. A period of sixty days was allotted for these treatments. Untreated controls were run with each series. Before employing the chemical salts, a period of three days was allowed for seedlings to recover sufficiently from the transplantings. All had recovered by the end of this period.

PART I: DETERMINATION OF THE MINIMUM TOXIC CONCENTRATIONS

Five seedlings not exceeding 3 cm. in height were placed into each of 50 glazed jars. Twenty-five of these jars were used for soil cultures and 25 for quartz sand cultures. Each culture was denoted for convenience by a lot number, so that the soil cultures became lot 1 and the quartz sand cultures lot 2.

TABLE IV.—PART I, CHEMICAL TREATMENTS FOR DETERMINING THE MINIMUM TOXIC CONCENTRATION

Chemical Substance	Number of Seedlings Used	Concentrations: Per Liter of Solution[a]	Concentrations: Per Cent
Pyridine	50	5.00 cc.	0.5
		1.00 cc.	0.1
		0.50 cc.	0.05
		0.10 cc.	0.01
		0.01 cc.	0.001
Piperidine	50	5.00 cc.	0.5
		1.00 cc.	0.1
		0.50 cc.	0.05
		0.10 cc.	0.01
		0.01 cc.	0.001
Atropine sulfate	50	0.500 Gm.	0.05
		0.250 Gm.	0.025
		0.100 Gm.	0.010
		0.005 Gm.	0.0005
		0.001 Gm.	0.0001
Thiamin chloride	50	0.0250 Gm.	0.0025
		0.0100 Gm.	0.0010
		0.0050 Gm.	0.0005
		0.0010 Gm.	0.0001
		0.0025 Gm.	0.00025
Untreated (control)	50	.....	.....

[a] Distilled water was used for seedlings of lot 1. Nutrient solution was used for seedlings of lot 2.

[1] The authors are indebted to Dr. W. R. Lloyd for the photographic records of the treatments and to Dr. C. V. Netz for photographic film strips made of the experiment.

Varying concentrations of the chemicals to be used in lot 1 were made with distilled water (Table IV). Daily waterings using 150 cc. of each concentration were then applied. One liter of freshly prepared nutrient solution with varying concentrations of the same chemicals was added daily to each of the quartz sand cultures. The cultures were thoroughly drained before new treatments were made.

Pyridine in Soil Cultures (Lot 1).—A definite growth response was evident for all seedlings receiving different concentrations of pyridine C. P. This was noted in particular for stem elongations. Stem measurements showed an increase in growth from 1 to 2 in. and more for a period of eight days. Following this period those seedlings receiving high concentrations, such as 0.5% and 0.1% solutions, showed a tendency of retarded growth. Two plants developed epinasty of the leaves from which they did not recover. Cotyledonary leaves dropped off as early as the third day of treatment. Plants receiving 0.05%, 0.01% and 0.001% solutions remained vigorous for a period of eighteen days. Following this period these plants failed to grow as actively as did the untreated controls. It was decided to continue the seedlings of Part II with a 0.01% concentration of pyridine.

Piperidine in Soil Cultures (Lot 1).—The response to varying concentrations of piperidine C. P. was nearly uniform for a period of ten days. Those seedlings receiving concentrations greater than 0.01% were slightly retarded in growth. No signs of epinasty of top growth were observed. The growth measurements of stem elongations varied from 1 to 2 in. for the period of the first ten days of feedings. Following this period the seedlings showed a sudden definite retardation of growth. All survived, however (Fig. 1). A 0.01% concentration was used for Part II.

Atropine Sulfate in Soil Cultures (Lot 1).—A favorable growth response was noted for concentrations below 0.05% during a period of twenty days of treatments (Fig. 1). Seedlings treated with a range of concentrations below this strength responded better than did those for either pyridine or piperidine (Fig. 1). Growth compared favorably with that of the untreated controls. Stem elongations varied from 1 to $3^1/_2$ in. The best response was with concentrations of 0.0005% and 0.0001%. Leaves became broader with concentrations of this strength. Two seedlings treated with 0.05% and 0.025% showed a marked roughening of the leaves. This roughening resembled that reported by Blakeslee (1) for colchicine-treated stramonium seedlings. Seedlings treated with a 0.01% concentration surpassed the piperidine responses but did not equal those of the untreated controls (Fig. 1). This per cent solution was used for continuing seedlings with treatments in Part II.

Thiamin Chloride in Soil Cultures (Lot 1).—The twenty-day growth response of seedlings treated with varying concentrations of vitamin B_1 as thiamin chloride was not uniform (Fig. 1). A greater response was observed among seedlings treated with concentrations lower than 0.250 Gm. per liter of solution. Stem elongations of these cultures measured from 2 to 5 in. Stem elongations ranged from $^1/_2$ to $1^1/_2$ in. longer than for the untreated controls and from 1 to 3 in. longer than for pyridine and piperidine treatments in twenty days. Leaf growth and stem growth at the concentrations of 0.001% and lower were found most satisfactory.

Controls in Soil Cultures (Lot 1).—Seedlings grown in untreated soil cultures (Fig. 1) and watered once every three days reached an average height of 3 to 4 in. for the period of twenty days. Leaf and stem growth was generally better than for pyridine, piperidine and atropine sulfate treatments. Stem elongations were slightly less than for thiamin chloride treatments of low concentration, but greater than those at strengths above 0.025%.

Pyridine in Quartz Sand Cultures (Lot 2).—Seedlings receiving 0.5% and 0.1% pyridine in nutrient solution for a period of eight days showed almost as vigorous a net growth response as did the controls

Fig. 1.—*Datura Stramonium* seedlings (Part I) after twenty days of daily feedings of the following chemicals added to soil cultures: (*A*) atropine sulfate in 0.05% to 0.0001% concentration, (*B*) untreated controls, (*C*) pyridine in 0.5% to 0.001% concentration, (*D*) piperidine in 0.5% to 0.001% concentration and (*E*) thiamin chloride in 0.0025% to 0.00025% concentration.

modified formula devised from the latter was found best suited for use. This nutrient formula is given in the following tables.

TABLE I.—NUTRIENT FORMULA

	Concentration, Cc./L.
Ammonium acid phosphate, 1 *M*	1
Calcium nitrate, 1 *M*	4
Potassium nitrate, 1 *M*	6
Magnesium sulfate, 1 *M*	2

To this solution a supplementary solution supplying boron, manganese, zinc, copper and molybdenum was added (Table II).

TABLE II.—SUPPLEMENTARY SOLUTION

	Concentration, Gm./L.
Boric acid	2.86
Manganese chloride	1.81
Zinc sulfate	0.22
Copper sulfate	0.08
Molybdic acid	0.02

One cubic centimeter of this solution (Table II) was added for each liter of nutrient solution at frequent intervals. This resulted in the concentrations shown in Table III.

TABLE III.—APPROXIMATE CONCENTRATIONS

	Parts per Million of Nutrient Solution
Boron	0.5
Manganese	0.5
Zinc	0.05
Copper	0.02
Molybdenum	0.01

In order to supply necessary iron a 0.5% iron tartrate solution was added about once a week to the nutrient cultures. Additions of this depended upon the chlorotic appearance of the plants. The *p*H of the solution was adjusted to approximately *p*H 6.

Quartz sand used in the experiment was supplied by the Minnesota Quartz Company, Mendota, Minnesota, and was assayed by the University of Minnesota Geology Department. It consisted of 99.6% of SiO_2 and 0.4% of limonite, calcite and iron. This was thoroughly washed before using.

Growing seedlings approximately 2 and 3 cm. in height, without the appearance of the young leaves but with two cotyledonary leaves, were transplanted from soil to each of a series of soil- and quartz sand-containing jars, and treated under glass. Two-gallon glazed earthenware jars were used. Each was equipped with suitable drainage systems.

The experiment[1] was divided into two parts: Part I consisted of the treatment of seedlings with five different concentrations of each of four chemical substances. The purpose of these treatments was to determine the minimum toxic concentration, or that concentration at which plants would show definite toxic effects when regularly saturated with the chemicals. A period of twenty days was allotted for these treatments. Part II consisted of treating quartz sand and soil cultures with a single concentration of each of the four chemical substances. This concentration was chosen only after the results of Part I could be ascertained and was based on these findings. Saturations were made once every three days for the soil cultures and twice weekly for the sand cultures of Part II. A period of sixty days was allotted for these treatments. Untreated controls were run with each series. Before employing the chemical salts, a period of three days was allowed for seedlings to recover sufficiently from the transplantings. All had recovered by the end of this period.

PART I: DETERMINATION OF THE MINIMUM TOXIC CONCENTRATIONS

Five seedlings not exceeding 3 cm. in height were placed into each of 50 glazed jars. Twenty-five of these jars were used for soil cultures and 25 for quartz sand cultures. Each culture was denoted for convenience by a lot number, so that the soil cultures became lot 1 and the quartz sand cultures lot 2.

TABLE IV.—PART I, CHEMICAL TREATMENTS FOR DETERMINING THE MINIMUM TOXIC CONCENTRATION

Chemical Substance	Number of Seedlings Used	Concentrations: Per Liter of Solution[a]	Concentrations: Per Cent
Pyridine	50	5.00 cc.	0.5
		1.00 cc.	0.1
		0.50 cc.	0.05
		0.10 cc.	0.01
		0.01 cc.	0.001
Piperidine	50	5.00 cc.	0.5
		1.00 cc.	0.1
		0.50 cc.	0.05
		0.10 cc.	0.01
		0.01 cc.	0.001
Atropine sulfate	50	0.500 Gm.	0.05
		0.250 Gm.	0.025
		0.100 Gm.	0.010
		0.005 Gm.	0.0005
		0.001 Gm.	0.0001
Thiamin chloride	50	0.0250 Gm.	0.0025
		0.0100 Gm.	0.0010
		0.0050 Gm.	0.0005
		0.0010 Gm.	0.0001
		0.0025 Gm.	0.00025
Untreated (control)	50	.....	.....

[a] Distilled water was used for seedlings of lot 1. Nutrient solution was used for seedlings of lot 2.

[1] The authors are indebted to Dr. W. R. Lloyd for the photographic records of the treatments and to Dr. C. V. Netz for photographic film strips made of the experiment.

Varying concentrations of the chemicals to be used in lot 1 were made with distilled water (Table IV). Daily waterings using 150 cc. of each concentration were then applied. One liter of freshly prepared nutrient solution with varying concentrations of the same chemicals was added daily to each of the quartz sand cultures. The cultures were thoroughly drained before new treatments were made.

Pyridine in Soil Cultures (Lot 1).—A definite growth response was evident for all seedlings receiving different concentrations of pyridine C. P. This was noted in particular for stem elongations. Stem measurements showed an increase in growth from 1 to 2 in. and more for a period of eight days. Following this period those seedlings receiving high concentrations, such as 0.5% and 0.1% solutions, showed a tendency of retarded growth. Two plants developed epinasty of the leaves from which they did not recover. Cotyledonary leaves dropped off as early as the third day of treatment. Plants receiving 0.05%, 0.01% and 0.001% solutions remained vigorous for a period of eighteen days. Following this period these plants failed to grow as actively as did the untreated controls. It was decided to continue the seedlings of Part II with a 0.01% concentration of pyridine.

Piperidine in Soil Cultures (Lot 1).—The response to varying concentrations of piperidine C. P. was nearly uniform for a period of ten days. Those seedlings receiving concentrations greater than 0.01% were slightly retarded in growth. No signs of epinasty of top growth were observed. The growth measurements of stem elongations varied from 1 to 2 in. for the period of the first ten days of feedings. Following this period the seedlings showed a sudden definite retardation of growth. All survived, however (Fig. 1). A 0.01% concentration was used for Part II.

Atropine Sulfate in Soil Cultures (Lot 1).—A favorable growth response was noted for concentrations below 0.05% during a period of twenty days of treatments (Fig. 1). Seedlings treated with a range of concentrations below this strength responded better than did those for either pyridine or piperidine (Fig. 1). Growth compared favorably with that of the untreated controls. Stem elongations varied from 1 to $3^1/_2$ in. The best response was with concentrations of 0.0005% and 0.0001%. Leaves became broader with concentrations of this strength. Two seedlings treated with 0.05% and 0.025% showed a marked roughening of the leaves. This roughening resembled that reported by Blakeslee (1) for colchicine-treated stramonium seedlings. Seedlings treated with a 0.01% concentration surpassed the piperidine responses but did not equal those of the untreated controls (Fig. 1). This per cent solution was used for continuing seedlings with treatments in Part II.

Thiamin Chloride in Soil Cultures (Lot 1).—The twenty-day growth response of seedlings treated with varying concentrations of vitamin B_1 as thiamin chloride was not uniform (Fig. 1). A greater response was observed among seedlings treated with concentrations lower than 0.250 Gm. per liter of solution. Stem elongations of these cultures measured from 2 to 5 in. Stem elongations ranged from $^1/_2$ to $1^1/_2$ in. longer than for the untreated controls and from 1 to 3 in. longer than for pyridine and piperidine treatments in twenty days. Leaf growth and stem growth at the concentrations of 0.001% and lower were found most satisfactory.

Controls in Soil Cultures (Lot 1).—Seedlings grown in untreated soil cultures (Fig. 1) and watered once every three days reached an average height of 3 to 4 in. for the period of twenty days. Leaf and stem growth was generally better than for pyridine, piperidine and atropine sulfate treatments. Stem elongations were slightly less than for thiamin chloride treatments of low concentration, but greater than those at strengths above 0.025%.

Pyridine in Quartz Sand Cultures (Lot 2).—Seedlings receiving 0.5% and 0.1% pyridine in nutrient solution for a period of eight days showed almost as vigorous a net growth response as did the controls

Fig. 1.—*Datura Stramonium* seedlings (Part I) after twenty days of daily feedings of the following chemicals added to soil cultures: (*A*) atropine sulfate in 0.05% to 0.0001% concentration, (*B*) untreated controls, (*C*) pyridine in 0.5% to 0.001% concentration, (*D*) piperidine in 0.5% to 0.001% concentration and (*E*) thiamin chloride in 0.0025% to 0.00025% concentration.

Fig. 2.—*Datura Stramonium* seedlings (Part I). Growth response of quartz sand cultures to (*A*) thiamin chloride (twenty days), (*B*) pyridine (twenty days), (*C*) thiamin chloride (eight days) and (*D*) pyridine (eight days). Concentrations given in Table IV.

Fig. 3.—*Datura Stramonium* seedlings (Part I). Growth response of quartz sand cultures to piperidine in 0.5% to 0.001% concentration (*A*) after eight days and (*B*) after twenty days of daily feedings.

(Fig. 2). Cotyledonary leaves dropped off soon after treatments were started. Three plants developed a roughening of the leaves from which they recovered. Most seedlings receiving 0.5% solutions showed an epinastic condition following eight days of feedings (Fig. 2). Lower concentrations such as 0.05%, 0.01% and 0.001% responded favorably to the twenty-day treatments. Concentrations of 0.05% and 0.01% showed the best stem elongation and leaf development. This compared satisfactorily with that of the nutrient control. A 0.01% concentration was adopted for Part II treatments.

Piperidine in Quartz Sand Cultures (Lot 2).—All seedings responded favorably to this treatment in nutrient solution (Fig. 3). A definite stem elongation was evident. This measured from 1 to 2 in. for a period of eight days. The growth was slightly slower among seedlings treated with higher concentrations (above 0.05%). No toxic responses were observed.

Atropine Sulfate in Quartz Sand Cultures (Lot 2).—Plants in nutrient solutions responded vigorously to the addition of all concentrations of atropine sulfate used. Stem elongations ranged from 2 to 4 in. for a twenty-day period. Leaf curling was observed among some of the plants receiving concentrations above 0.05%. Three of the 25 seedlings showed a slight degree of epinasty following twelve days of feedings. A concentration of 0.01% was chosen for Part II treatments.

Thiamin Chloride in Quartz Sand Cultures (Lot 2).—Concentrations lower than 0.025 Gm. (0.0025%) in nutrient solution produced a vigorous growth for the twenty-day period (Fig. 2). Stem elongation was rapid and surpassed most of the nutrient control seedlings. The lowest concentrations produced the best response. High concentrations, above 0.002%, remained less active until after approximately ten days of treatment. Measurements of 4 and 5 in. were recorded for plant growth. A concentration of 0.001% was chosen for Part II.

Controls in Quartz Sand Cultures (Lot 2).—The twenty-day growth response of seedlings regularly treated with fresh nutrient solution (Tables I, II and III) was in general slightly greater than for this solution with each of the four chemical substances added. A less vigorous response was noted when compared with vitamin B_1 treatments.

PART II: SIXTY-DAY CONTINUOUS TREATMENTS

This portion of the experiment was carried out in much the same fashion as Part I. Concentrations now used were selected from the established results of growth responses in Part I. It was decided to select as closely as possible similar concentrations in order that a correlation on this basis might also be determined. Two seedlings not exceeding 3 cm. in height were placed into each of 50 glazed jars and a suitable time allowed for recovery from transplanting. Soil cultures were classed as lot 1 and quartz sand cultures as lot 2. Soil cultures were saturated once every three days with chemical solutions in distilled water. Sand cultures were completely changed twice a week with freshly prepared chemicals in nutrient solution. These treatments were continued from July 10 to September 9. Concentrations of chemical substances used are tabulated in Table V.

TABLE V.—PART II, CHEMICAL TREATMENTS FOR SIXTY-DAY PERIOD

Chemical Substance	Number of Seedlings Used	Concentration Per Liter of Solution[a]	Per Cent
Pyridine	10	0.1 cc.	0.01
Piperidine	10	0.1 cc.	0.01
Atropine sulfate	10	0.1 Gm.	0.01
Thiamin chloride	10	0.01 Gm.	0.001
Untreated (control)	10	.....	.....

[a] Distilled water was used for seedlings of lot 1. Nutrient solution was used for seedlings of lot 2.

Pyridine in Soil Cultures (Lot 1).—Growth responses with a 0.01% solution of pyridine were slow from the beginning of the sixty-day period. After thirty days of feeding most seedlings reached a height of about $6^1/_2$ in. Leaves developed normally without any evident epinasty. The width of all leaves examined equaled that of the control plants. Following forty-five days of treatments stem elongation measured approximately 2 in. shorter than that of the untreated seedlings. A greater increase was noted in width of leaves, but little stem elongation continued. In sixty days most plants had reached an average height of 10 in., but with well-developed stems and leaves (Fig. 4). No apparent toxicity could be ascertained. Flower buds developed on many of the plants.

Piperidine in Soil Cultures (Lot 1).—A 0.01% solution of piperidine fed once every three days to soil cultures produced as vigorous a response as that for pyridine. The stem elongation of most plants equaled that of the untreated soil control for the first six days of feedings. Following this period stem elongations were slower and became generally from one-half to three-fourths the size of the untreated controls for a period of twenty-five days. Plants reached a height of nearly 6 in. at the end of thirty days. During the last week of treatment some of the leaves developed white spots. None of the plants failed to survive. A maximum stem elongation of 12 in. was recorded for several plants (Fig. 4). This was about 6 in. shorter than the average stem elongation of the untreated controls. The shortest plant measured $6^1/_2$ in. Leaves were well developed and a few floral buds developed on the more mature plants.

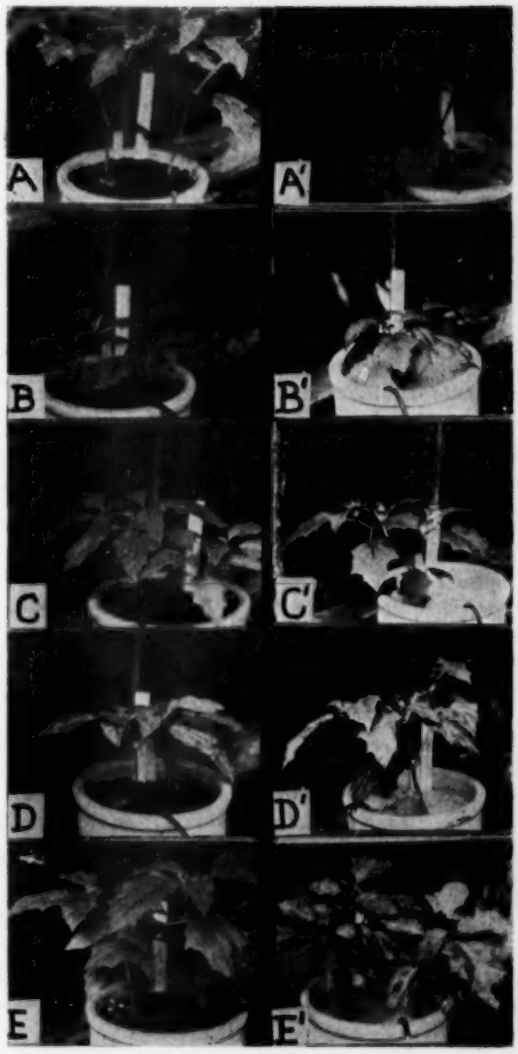


Fig. 4.—*Datura Stramonium* seedlings (Part II). Growth response after sixty days of feedings (left, soil cultures; right, quartz sand cultures): (*A*, *A'*) untreated controls, (*B*, *B'*) pyridine, (*C*, *C'*) piperidine, (*D*, *D'*) atropine sulfate and (*E*, *E'*) thiamin chloride. Concentrations given in Table V.

Atropine Sulfate in Soil Cultures (Lot 1).—Shortly following the first twenty days of feedings in soil

cultures with atropine sulfate (a 0.01% solution), stem elongations suddenly exhibited a retarded growth. In thirty days this elongation became notedly less than that of the control plants. Most stems did not increase in length after this period by more than $3^1/_2$ in. The leaves of three plants showed a marked roughening (see Part I, lot 1). Two of the plants recovered from this condition. Several plants failed to recover from a condition of epinasty and white marks (Fig. 4) developed on the leaves of four plants. The leaves of most of the surviving plants developed as large a growth as the untreated controls (Fig. 4).

Thiamin Chloride in Soil Cultures (Lot 1).—Treatments of 0.001% thiamin chloride once every three days produced vigorous growth. Stem elongations ranged from 2 to $4^1/_2$ in. in eight days of feedings. At the end of thirty days most seedlings had attained a size of from 4 to $6^1/_2$ in. In forty-five days of treatments growth reached 12 in. and at the conclusion of the experiment average stem elongations (Fig. 4) were about 15 in. No plants developed white marks or epinasty. Flower buds were numerous and the leaves of all plants were seemingly more vigorous than those developed on control untreated plants.

Controls in Soil Cultures (Lot 1).—These plants were treated with tap water once every three days. Moisture accumulating in the jars was allowed to drain in the same manner as in the chemical treatments. Steam elongations ranged from $2^1/_2$ to 5 in. in a twenty-day period and reached an average height of 7 in. in thirty days. At the conclusion of sixty days of treatments the average size of the untreated control plants was from 15 to 18 in., (Fig. 4). No epinasty or leaf curling developed among the untreated plants. Flower buds were numerous.

Pyridine in Quartz Sand Cultures (Lot 2).—Seedlings in sand cultures treated twice weekly with a 0.01% pyridine in nutrient solution showed a slow growth response. Shortly following the start of the treatments several plants showed epinasty of the leaves. Most recovered from this; and stem elongations of 6 in., $2^1/_2$ in. shorter than for the untreated quartz sand control plants, were observed at the end of thirty days. The growth seemed to be concentrated in the leaves. In sixty days of feedings the maximum stem elongation was 8 in. (Fig. 4). This was about one-half the size of the nutrient untreated controls. Flower buds developed on four of the six remaining plants.

Piperidine in Quartz Sand Cultures (Lot 2).—Sand cultures treated with a 0.01% piperidine in nutrient solution exhibited a growth response similar to that of the nutrient quartz sand control plants. Stem elongations and leaf size were on the average larger than for pyridine-treated seedlings of lot 2. In thirty days several stem elongations of 7 and 8 in. were recorded. Growth continued at a slower rate than that of the untreated controls following the next five days of feedings. In sixty days (Fig. 4) elongations of 11 and 12 in. were observed. These growth measurements equaled approximately three-fourths those of the controls. Several flower buds were formed. One plant after forty days of treatments developed a marked roughening of the leaves. It did not recover completely from this, but did bear flower buds.

Atropine Sulfate in Quartz Sand Cultures (Lot 2).—A 0.01% concentration of atropine sulfate in nutrient solution produced a favorable growth response during the early feedings. Stem elongations and leaf developments were as fast as those of the controls at first. However, following the first thirty days of treatments this growth became noticeably slower. Four plants showed an epinastic condition but recovered from it. At the end of this period stem elongations measured from 4 to $7^1/_2$ in. This equaled the average size as recorded for the nutrient control plants for the same period. After sixty days of feedings (Fig. 4) plants measured from 10 to 12 in. in height or almost 4 in. less than the control plants. Many flower buds were produced and some plants showed white marks on the leaves. Leaves compared favorably in size with the controls.

Thiamin Chloride in Quartz Sand Cultures (Lot 2).—A very noticeable increase in growth was noted for seedlings treated with 0.001% thiamin chloride in nutrient solution. Stem elongations of from 5 to $8^1/_2$ in. were noted in thirty days of continuous feeding. This was increased to from 10 to 17 in. in sixty days (Fig. 4). No epinasty developed. No signs of leaf curling were evident. White marks did occur on some of the more mature leaves (Fig. 4) during the final week of the treatments. Flower buds developed on eight of the ten plants.

Control Seedlings in Quartz Sand Cultures (Lot 2).—A modified nutrient solution (Tables I, II and III) was added to each jar in the same manner as for the chemical treatments. Following twenty days of growth, stem elongations measured up to 5 in. for most of the control plants. This increased to $9^1/_2$ in. at the end of thirty days of treatment. The average untreated control attained a height of 16 in. (Fig. 4) at the conclusion of the experiment. No epinasty or leaf roughening was noticed. White marks did develop on the leaves of a few plants during the final six days of growth. In general, control untreated plants (Fig. 4) grew larger in sand cultures than did the chemically treated plants in the same culture media. They attained a height and leaf growth only slightly less than plants treated with vitamin B_1 as thiamin chloride.

SUMMARY

Several concentrations of chemically pure pyridine, piperidine, atropine sulfate and thiamin chloride have been applied in continuous feedings to growing stramonium seedlings in soil and pure quartz sand cultures.

In general, the lower the concentration of these chemical substances, the better was the growth response. Concentrations of 0.025% or greater were found to produce the most toxic effects.

Pyridine, piperidine and atropine sulfate induced marked inhibitory stem growth responses during a period of sixty-day treatments. However, leaf growth was not appreciably impaired during the same period. A condition of roughened leaves has been described for some seedlings treated with piperidine and atropine sulfate. This response was found to be similar to that previously reported for stramonium seedlings treated with colchicine (1).

Epinastic responses and other toxic manifestations varied according to the size and the rate of growth of the seedlings.

Thiamin chloride accelerated growth responses. Concentrations of 0.001% and 0.0025% produced the most vigorous growth activity.

The fact that stramonium plants are influenced to some extent in stem and leaf growth by those substances which have a chemical structure related to the atropine nucleus and that they can tolerate small concentrations of these chemicals indicates the possibility that by feeding the plant continuous tolerated doses, the alkaloid content in the leaves might also be influenced. This phase of the experiment is being continued and will be reported subsequently.

REFERENCES

(1) Blakeslee, A. F., and Avery, A. G., *J. Heredity*, 28 (1937), 393.
(2) Avery, G. S., *et al.*, *Am. J. Botany*, 24 (1937), 553.
(3) Zopf, L. C., JOUR. A. PH. A., 29 (1940), 488.
(4) Nicol, H., "Plant Growth Substances," Leonard Hill, London, 1938.
(5) Hoagland, D. R., and Arnon, D. I., Univ Calif. Coll. Agr. Circ. 347, December, 1938.

A Note on the Healing Action of Allantoin, Allantoin Dipiperazine and Urea*

Preliminary Report

By Frederick R. Greenbaum

The reintroduction of allantoin clinically occurred in 1935 through the work of Robinson (1) who demonstrated that one of the active principles of the excretion of maggots is allantoin. Greenbaum (2) reported on the healing action of allantoin in various clinical cases. In another publication by Robinson (3) it was shown that urea is present in the extracts of maggots, and that it also possesses healing action. Since this publication appeared, several investigators have tried urea to stimulate healing in chronic purulent wounds and in infected wounds (4, 5, 6, 7).

Urea is inexpensive, and therefore some clinicians might prefer to use urea provided it is as effective as allantoin. Holder and Mackey (7) used a 40% urea solution and also crystals of urea. Forty per cent urea solution has a tendency to dry and cake on occasion, and there is difficulty in maintaining it in proper contact with the tissue at times. The use of concentrated urea solution is frequently accompanied with pain so pronounced that it is necessary that a local anesthetic be added to the concentrated urea solution.

EXPERIMENTAL

The purpose of this investigation was to demonstrate by means of animal experiments the relative healing properties of allantoin and urea. We also included in this investigation a combination of allantoin with piperazine, which is more soluble than allantoin. This combination is a double salt of allantoin with piperazine. It was prepared by dissolving 2 moles of piperazine in water and 1 mole of allantoin. The maximum solubility of

* This work was carried out at the Research Laboratories of the National Drug Company, Philadelphia, Pa.

Table I

Animal	Weight	Nature of Burn	—Time of Healing in Days of Burns Treated with— Allantoin	Saline Solution	Allantoin Dipiperazine	40% Urea	Remarks
Rabbits, 2	2 Kg.	Hot soldering iron	13	13	13	..	Granulation occurred after 5 days
Rabbits, 2	2 Kg.	Hot soldering iron	14	14	14	..	In 2 days showed signs of repair
Rabbits, 2	2 Kg.	Sodium hydroxide	8[a]	..	..	..	Rabbits died
Rabbits, 4	2 Kg.	Sodium hydroxide	25	25	27	30	
Pigeons, 2	...	Sodium hydroxide	14	21	..	..	
Roosters, 4	3 Lb.	Sodium hydroxide	14	19	20	27	

[a] Evidence of healing.

allantoin at room temperature is 0.6%. Piperazine increases the solubility so that 6% of allantoin remains in solution at room temperature.

Our attention was directed to a paper by Milles and Farley (8) who studied a comparison of the effects of an antiseptic jelly, with and without larvæ ingredients, on burns produced with a red-hot iron. These workers showed on a group of rabbits the superiority of healing obtained with a jelly containing comminuted larvæ *Lucilia sericata* over a plain jelly without the larvæ material.

The study here reported is our observation concerning the comparative healing action of allantoin and allantoin dipiperazine on experimental animals.

Table I gives a short account of the healing action of allantoin, saline solution, allantoin dipiperazine and 40% urea. The time given indicates when complete healing occurred.

DISCUSSION OF RESULTS

It was demonstrated on a very limited number of animals in a very preliminary way that the healing action of allantoin is superior to that of allantoin dipiperazine. However, under our experimental conditions, rabbits are not entirely suitable for this type of experiment due to the fact that they are fur-bearing animals.

Using pigeons, the definite healing action of allantoin solution and of allantoin ointment was again demonstrated, but pigeons are rather small for this test.

We therefore selected roosters and found them more suitable for this study. It was in the roosters that we demonstrated that allantoin has the best and quickest healing action, completely healing a chemical burn in a rooster in 14 days. In the control bird that was treated with saline solution, the healing required 19 days; with allantoin dipiperazine, 20 days; and with 40% urea solution, 27 days. These experiments clearly show that 40% urea solution greatly retards the healing. It also shows that the allantoin dipiperazine is inferior to the plain allantoin. The explanation of this behavior is, however, in complete agreement with the chemical nature of this compound of allantoin. It has been found that an allantoin dipiperazine solution has a greater solubility than allantoin and the solution thus obtained is very alkaline in nature (*p*H 11 or more). Therefore, as the high *p*H destroys the allantoin, allantoin dipiperazine is decomposed in solution and most of the allantoin is chemically broken down, and therefore loses its healing potency.

The healing action of allantoin is based on the stimulation of epithelial growth, while the healing action of urea is based on the removal of devitalized tissue.

Macalister (9) offers the theory that allantoin may possibly have something to do with the activity of nucleic acid. In the chemical makeup of nucleic acid, we have a phosphoric acid residue linked to a pentose sugar and to adenine, thymine, guanine, or cytosine. These latter substances are purine bodies, and it is to these purine bodies that allantoin may be indirectly related since purine bodies yield uric acid in the course of their chemical metabolism, while uric acid when suitably oxidized yields allantoin.

It seems reasonable to suppose that cellular stability may depend upon the efficient building up of nucleic acid. The connection between nucleic acid and allantoin is, however, a very indirect one. This is only a hypothesis; no definite proof has been furnished.

There is some evidence, as reported by Greenbaum (10), that the administration of allantoin produces leucocytosis in animals. This opens up the possibility that allantoin exerts its healing action partly through a local leucocytosis which sets in at the site of the wound.

Much more research with a greater number of animals is necessary before any definite conclusions may be drawn.

SUMMARY

1. Rabbits are fur-bearing animals, and, therefore, are not satisfactory animals, under our experimental conditions, to test comparatively the healing action of various proliferative substances.

2. Pigeons, on the other hand, are birds

which are too small to be profitably used for this type of experimentation.

3. Roosters appear to be suitable for this type of study.

4. While the number of animals is undoubtedly very limited, it has been shown that of the four agents studied—allantoin, allantoin dipiperazine, 40% urea and physiologic salt solution—allantoin possesses the best healing action, urea the least.

5. Allantoin dipiperazine shows no better healing or granulating action than saline solution because the alkalinity of the solution destroys the allantoin, and thus the potency.

REFERENCES

(1) Robinson, W., *J. Bone Joint Surg.*, 18 (1935), 267.

(2) Greenbaum, F. R., *Am. J. Surg.*, 34 (1936), 259.

(3) Robinson, W., *Ibid.*, 33 (1936), 192.

(4) Holder, H. G., and Mackey, E. M., *J. Am. Med. Assoc.*, 108 (1937), 1167.

(5) Baker, G. E., *Rocky Mountain Med. J.* (April, 1938).

(6) Muldavin, L. R., and Holtzman, J. M., *J. Am. Med. Assoc.*, 110 (1938), 1879.

(7) Holder, H. G., and Mackey, E. M., *Ann. Surg.* (1939), p. 110.

(8) Milles, G., and Farley, R. T., *Am. J. Surg.*, 45 (1939), 76.

(9) Macalister, C. J., "The *Symphytum officinale* and Its Contained Allantoin," John Babe, Sons & Danielson, Ltd., London, 1936.

(10) Greenbaum, F. R., *Med. Record* (April 17, 1940).

A Study of Syrup of Hydriodic Acid*

By Howard Hopkins† and C. O. Lee‡

The solution of hydriodic acid from which the syrup was first prepared was unstable due to the presence of potassium iodate in the potassium iodide. Discoloration of the syrup in years gone by may have been due to the liberation of free iodine. With the coming of more highly purified chemicals its discoloration has been ascribed to other causes. Since about 1880 there have been numerous comments and discussions by many workers on how best to prepare, stabilize and store syrup of hydriodic acid.

Hydriodic acid has been used in medicine for more than a hundred years. Because of its unstable character it has been a problem. Many studies have been made with the view to finding a more suitable method for its preparation and preservation.

The first to report the use of hydriodic acid as a medicinal was Dr. Andrew Buchanan, Junior Surgeon to the Glasgow Royal Infirmary in 1837 (1, 2, 3). He gave detailed directions for the preparation of a dilute solution of the acid by the interaction of potassium iodide and tartaric acid. He preferred to use the acid, in the place of iodine, because he believed that it was less irritating to the stomach. He believed, also, that the stomach converted iodine into hydriodic acid, which reaction would be saved by administering the latter preparation. Buchanan proposed that the acid should be taken with starch gruel, arguing that any iodine which might be liberated would combine with the starch, and be less irritating.

Apparently the first attempt to stabilize the solution of hydriodic acid was reported in 1855 by Murdock (3). He said, "I find that hydriodic acid may be prevented from undergoing this decomposition when in the form of a syrup." He said further, "Assuming, therefore, that if a syrup can be prepared by Dr. Buchanan's solution that shall contain no free iodine, it will furnish the most suitable manner of obtaining this acid for medicinal purposes....It is necessary, however, to observe, as one of the conditions of success, that the iodide must be free from any trace of iodate of potash."

The U. S. Pharmacopœia IV (4) included a formula for diluted hydriodic acid, and

* Presented to the Section on Practical Pharmacy of the A. PH. A., Detroit meeting, 1941.

† Dean of Pharmacy, Ferris Institute, Big Rapids, Mich.

‡ Professor of Pharmacy, Purdue University.

directed that it be prepared by suspending iodine in water and passing hydrogen sulfide into the suspension. The U. S. Pharmacopœias V and VI offered no formulas for hydriodic acid. However the latter book (5) contained a formula for syrup of hydriodic acid, to be prepared according to the method of the U. S. Pharmacopœia IV. It was directed that the finished syrup be put into small, securely corked vials, and kept in a cool, dark place.

REPORTS OF EARLY INVESTIGATORS

In 1882 Godding (6) submitted a thesis to the Massachusetts College of Pharmacy in which were his findings concerning his efforts to stabilize syrup of hydriodic acid. He says, "It has been asserted that hyposulphite of sodium in solution has the advantage of preserving and reclaiming the acid when colored, but my experiments have failed to prove this assertion." Hence, this early attempt to prevent the discoloration that develops in syrup of hydriodic acid was not successful. Since Godding's time many investigators have given thought to this problem.

Cameron (7) proposed that the acid be prepared frequently, added to syrup, and stored in a cool, dark place. He said that syrup was the best vehicle available, since it prevented the discoloration so frequently observed in diluted hydriodic acid.

The first edition of the National Formulary (8), 1888, contained a formula for colorless syrup of hydriodic acid. The acid was prepared according to a modification of Buchanan's method, added to syrup, and preserved by the addition of potassium hypophosphite. So far as we know, this was the first time that this salt was proposed as a preservative for syrup of hydriodic acid.

In 1889 Beringer (9) was reported to have said that he had prepared a syrup of hydriodic acid which had remained stable for two years. Concerning the acid, England (10) said, "Since its introduction it has found a limited and varying demand among the medical profession." He attributed its lack of wide popularity to its instability. He claimed that honey, potassium hypophosphite, hypophosphorous acid and glycerin had little or no influence in retarding decomposition of the acid. He approved the National Formulary colorless syrup, but substituted syrupy glucose for the potassium hypophosphite. Raubenheimer (11) claimed that the National Formulary formula for colorless syrup of hydriodic acid was not so stable as his modification thereof containing glycerin.

The U. S. Pharmacopœia VII (12) did not include a diluted hydriodic acid, but the syrup was retained. The acid to be used in preparing the syrup was directed to be prepared according to a modification of Buchanan's method in preference to the hydrogen sulfide–iodine method, U. S. P. IV.

Syrup of hydriodic acid frequently developed an offensive odor upon standing. Haussmann (13) attributed this to the decomposition of ultramarine in acid media, hydrogen sulfide being liberated from ultramarine. Caspari (14) claimed that syrup of hydriodic acid kept well for some time if preserved in completely filled bottles and in a dark place.

In 1897 the U. S. Pharmacopœia Committee of Revision (15) reported that syrup of hydriodic acid was considered to be an unstable preparation and proposed that it be made extemporaneously from a strong solution of hydriodic acid and syrup.

Scoville (16) in 1899 reported that the more dilute the syrup, the more stable the preparation. He also reported that the substitution of glycerin for syrup yielded a stable product. Wentworth (17) confirmed this observation.

Peacock (18) proposed that the coloration observed in syrup of hydriodic acid might be due to caramelization of the sugar. Haussmann (19) concurred in this and suggested that the syrup be prepared by merely diluting the acid with simple syrup. He discounted the assertions of some that glycerin was an effective substitute for syrup. He indicated, however, that heat was of importance in producing discoloration of syrup of hydriodic acid. His investigations failed to reveal the presence of free iodine in discolored samples of syrup of hydriodic acid. The same samples were shown to

have lost none of their hydriodic acid content. Haussmann said, "A number of examinations revealed the fact that syrups at the time of preparation and after discoloration show identical percentages." He suggested the use of animal charcoal to remove color from the syrup, because it did not take out any of the hydriodic acid.

Wells (20) claimed that discoloration of syrup of hydriodic acid was, in nearly all cases, due to the action of the acid on impurities in the sugar or upon the sugar itself. In support of his contention he pointed out that other acid syrups, such as syrup of calcium lactophosphate, showed discoloration. He agreed with Scoville that a light syrup seemed more effective as a stabilizer than a heavy syrup. Inasmuch as heat caused syrup of hydriodic acid to discolor, he argued that it should be stored in a cool place.

Sieker (21) reported in 1902 that the discoloration or precipitate which developed in syrup of hydriodic acid was due to the decomposition of the sugars. He suggested that the syrup be prepared extemporaneously by diluting 1 vol. of 16% hydriodic acid with 15 vols. of simple syrup.

Koch (22) held to the view of Haussmann and Sieker that discoloration of the syrup of hydriodic acid was due to the decomposition of the sugar and agreed with Scoville and Wells that a light syrup was a more effective stabilizer than a heavy syrup.

The U. S. Pharmacopœia VIII (23) reintroduced diluted hydriodic acid. It was prepared according to a modification of Buchanan's method and the official syrup was made by mixing the diluted acid with simple syrup.

Francis (24) proposed that glycerin be used as the vehicle for hydriodic acid and that such a product be called "glycerole."

In 1908 Cook (25) showed that the use of a light syrup made a syrup of hydriodic acid which did not darken upon standing.

The U. S. Pharmacopœia IX (26) included diluted hydriodic acid and syrup prepared from it, the latter being 20% stronger than the formula in U. S. Pharmacopœia VIII.

In 1920 Snyder (27) reported for a committee of the American Drug Manufacturers Association to the effect that it believed that discoloration of syrup of hydriodic acid was due to the decomposition of a portion of the sugar. The committee recommended that the Pharmacopœia should state that the syrup should be made in small quantities and stored in a cool place.

Ewe (28) recommended a formula for syrup of hydriodic acid to contain 34 Gm. of sugar per 100 cc. of the syrup. He said that a higher concentration of sugar caused darkening and a lower one made an inferior product. Krantz (29) claimed that 72.5% of glycerin by volume was a better vehicle for hydriodic acid than syrup.

The U. S. Pharmacopœia X (30) included formulas for both diluted hydriodic acid and the syrup, the latter to be prepared from the diluted acid, sugar and distilled water.

ASSAYS OF SYRUP OF HYDRIODIC ACID

The first assay results to be reported upon the stability of hydriodic acid were those of Arny and his co-workers (31) in 1929. Their studies, which extended over nearly two years, showed that there was no loss of the iodide content in numerous samples of hydriodic acid made using glycerin, reducing sugars, and syrup in the formulas.

Snyder (32) expressed the view, in 1930, that syrup of hydriodic acid was a drugstore product that every pharmacist should be prepared to compound extemporaneously. He suggested that the syrup prepared from glycerin developed disagreeable odors which were attributed to the presence of impurities in the glycerin. Ewe (33) pointed out that glycerin would be a satisfactory vehicle for hydriodic acid if it were free from butyric acid. He believed that discoloration of the syrup was due to caramelization of the sugar by excessive acidity. Heyl (34) meanwhile reported that a reduction of the hypophosphorous content did not prevent precipitation and discoloration of the syrup. Saalbach (35) claimed that discolored syrups could be made colorless upon their exposure to direct sunlight. He further suggested that the Pharmacopœia should state

that it be stored in clear glass bottles exposed to the sunlight.

In 1931 Husa (36) reported the first of a series of studies upon hydriodic acid and syrup of hydriodic acid. It was his belief, at that time, that the syrup should be prepared in the drugstore as needed. In the same year Arny (37) and his co-workers reported their studies upon the deterioration of certain medicaments under the influence of various conditions of light and storage. They had the following to say, "The...products, all of the quality prescribed by the U. S. P. X or U. S. P. IX, maintained their full halogen content, even after one year of exposure to direct daylight or to diffused light.... The iodide preparations did darken in direct daylight, all samples darkening between the fourth and sixth month of exposure except in the case of flint glass and the amber containers where the sample of the acid remained colorless for about twelve months." They declared that hypophosphorous acid was mistakenly added as a preservative.

Husa and Klotz (38) attacked the problem from a new angle in 1935. They used different sugars in preparing the syrup of hydriodic acid and concluded that its discoloration was due to the decomposition of levulose formed upon hydrolysis of the sugar.

They were able to prepare a superior syrup by using 700 Gm. of dextrose (commercial or a chemically pure grade) per liter of the finished product. It remained clear and colorless even upon continued exposure to an oven temperature of 50° C. for three months.

EXPERIMENTAL

The experimental part of this study follows closely that of Husa and Klotz. The sugars sucrose and glucose were used alone and in combination. Glycerin and alcohol were used in the same way in part of the formulas, replacing an equal volume of water in each case.

Each sample of syrup was divided into three 2-oz. portions in regular prescription bottles. Each portion was stored differently; namely, (*a*) in a warm place (about 40° C.), (*b*) in a refrigerator and (*c*) at room temperature. Observations were made at frequent intervals. The samples were assayed at the time of preparation or soon thereafter, and at the end of the test period.

Twenty formulas were prepared as indicated in Table I.

The U. S. P. XI assay procedure was followed except that a smaller quantity of syrup than that

TABLE I

	Formulas and Variations			
	1*	2	3	4
Formula A				
Diluted HI	23.4 cc.	23.4 cc.	23.4 cc.	23.4 cc.
Sucrose	81.0 Gm.	81.0 Gm.	81.0 Gm.	81.0 Gm.
Glycerin	.....	18.0 cc.	.....	18.0 cc.
Alcohol	.....	.....	19.0 cc.	19.0 cc.
Dist. water, *q. s. ad.*	180.0 cc.	180.0 cc.	180.0 cc.	180.0 cc.
Formula B				
Diluted HI	23.4 cc.	23.4 cc.	23.4 cc.	23.4 cc.
Dextrose	126.0 Gm.	126.0 Gm.	126.0 Gm.	126.0 Gm.
Glycerin	.....	18.0 cc.	.....	18.0 cc.
Alcohol	.....	.....	19.0 cc.	19.0 cc.
Dist. water, *q. s. ad.*	180.0 cc.	180.0 cc.	180.0 cc.	180.0 cc.
Formula C				
Diluted HI	23.4 cc.	23.4 cc.	23.4 cc.	23.4 cc.
Glucose	78.3 cc.	78.3 cc.	78.3 cc.	78.3 cc.
Glycerin	.....	18.0 cc.	.....	18.0 cc.
Alcohol	.....	.....	19.0 cc.	19.0 cc.
Dist. water, *q. s. ad.*	180.0 cc.	180.0 cc.	180.0 cc.	180.0 cc.
Formula D				
Diluted HI	23.4 cc.	23.4 cc.	23.4 cc.	23.4 cc.
Sucrose	64.8 Gm.	56.7 Gm.	40.5 Gm.	24.3 Gm.
Dextrose	25.2 Gm.	37.8 Gm.	63.0 Gm.	88.2 Gm.
Dist. water, *q. s. ad.*	180.0 cc.	180.0 cc.	180.0 cc.	180.0 cc.
Formula E				
Diluted HI	23.4 cc.	23.4 cc.	23.4 cc.	23.4 cc.
Sucrose	86.4 Gm.	72.0 Gm.	61.2 Gm.	54.0 Gm.
Dist. water, *q. s. ad.*	180.0 cc.	180.0 cc.	180.0 cc.	180.0 cc.

* Formula 1-*A* official in U. S. P. XI.

specified was taken for a sample. Ten cubic centimeters of the syrup and 40 cc. of distilled water were placed in a 250-cc. Erlenmeyer flask and mixed well, 25.00 cc. of 0.1 *N* silver nitrate solution was added next, and lastly 2 cc. of nitric acid; the mixture was then placed on a water bath and heated until the precipitate acquired a yellow color or until the supernatant liquid became clarified. The excess silver nitrate was then titrated with 0.1 *N* ammonium thiocyanate solution. Ferric ammonium alum was used as the indicator. Duplicate assays were run in each instance. The assay results are given in Table II.

TABLE II

Date of Assay, 1941	Storage Condition	Grams of HI per 100 Cc. of Syrup 1	2	3	4
Formula A					
April 12	Cold	1.390	1.407	1.381	1.388
	Room	1.357	1.406	1.383	1.394[a]
	Warm	1.380	1.395	1.371	1.388
May 14	Cold	1.401	1.413	1.386	1.407
	Room	1.344	1.394	1.387	1.403
	Warm	1.367[a]	1.397	1.361	1.389
Formula B					
April 12	Cold	...	...	...	...
	Room	...	...	...	...
	Warm	...	...	...	...
May 14	Cold	1.386	1.501	1.373	1.377
	Room	1.382	1.500	1.372	1.385
	Warm	1.383	1.498	1.360	1.386
Formula C					
April 17	Cold	1.674	1.472	1.674	1.627
	Room	1.670	1.496	1.528	1.528
	Warm	1.528	1.496	1.528	1.550
May 14	Cold	1.542	1.500	1.542	1.544
	Room	1.564	1.486	1.550	1.542
	Warm	1.542	1.500	1.546	1.563
Formula D					
April 12	Cold	1.458	1.496	1.670	1.496
	Room	1.458	1.496	1.670	1.496
	Warm	1.458	1.496	1.670	1.496
May 14	Cold	1.489	1.516	1.558	1.516
	Room	1.462	1.518	1.500	1.500
	Warm	1.475	1.489	1.558	1.516
Formula E					
April 12	Cold	1.350	1.375	1.376	1.378
	Room	1.350	1.375	1.376	1.378
	Warm	1.350	1.375	1.376	1.378
May 14	Cold	1.377	1.382	1.410	1.377
	Room	1.374	1.377	1.402	1.365
	Warm	1.361	1.378	1.377	1.361

[a] A single determination.

From these results, it is evident that there is no significant loss of the iodide content when syrup of hydriodic acid is stored at room temperature, in the cold, or in a warm place. The conditions of storage, however, have a marked effect on the discoloration of the syrup. In general, it may be said that those samples stored in a warm place darkened quickly and to a marked degree, whereas those stored at room temperature darkened less quickly and to a lesser degree. The samples of syrup stored in the refrigerator did not, in general, darken at all.

Formula *A* gave syrups that darkened quickly and intensely. They contained sucrose alone as the sugar. Formula *E*, containing only sucrose as the sugar, produced syrups that gave increasing discoloration with increasing sugar concentration. Formulas *C* and *D* produced syrups that discolored somewhat. Formula *B* gave a syrup of hydriodic acid that remained clear and colorless in all instances. It was made with dextrose. These results coincide with those of Husa and Klotz. Their proposal to use dextrose instead of sucrose in preparing syrup of hydriodic acid seems to us to be sound.

SUMMARY AND CONCLUSIONS

1. The early history and development of the use of hydriodic acid and syrup of hydriodic acid have been reviewed.

2. Assay results are given showing that discoloration, if it occurs, does not affect the iodide concentration in syrup of hydriodic acid.

3. In general, the rate of discoloration and the intensity of the color produced increase as the storage temperature increases.

4. When sucrose alone is used as the sugar in preparing syrup of hydriodic acid, the rate of discoloration and the intensity of the color produced increase with increasing sucrose concentration.

REFERENCES

(1) *London Med. Gaz.*, 18 (1837), 517; through *Am. J. Pharm.*, 9 (1837), 175.

(2) Pereira, J., "The Elements of Materia Medica and Therapeutics," Third American edition, 1852, p. 405.

(3) Murdock, J., *Pharm. J.*, 15 (1855), 63.

(4) "The Pharmacopœia of the United States of America," Fourth revision, 1864, p. 71.

(5) *Ibid.*, Sixth revision, 1882, p. 318.

(6) Godding, E. R., *New Remedies* (April, 1882); through *Pharm. J.*, 41 (1882), 1035.

(7) Cameron, D. L., *Pharm. J.*, 47 (1887), 163.

(8) "National Formulary," First edition, 1888, p. 122.

(9) Weigand, T. S., *Am. J. Pharm.*, 61 (1889), 49.

(10) England, J. W., *Ibid.*, 61 (1889), 14.

(11) Raubenheimer, O., *Pharm. Era*, 3 (1889), 186.

(12) "The Pharmacopœia of the United States of America," Seventh revision, 1893, p. 388.

(13) Haussmann, F. W., *Am. J. Pharm.*, 67 (1895), 84.

(14) Caspari, C., Jr., "A Treatise on Pharmacy," 1895, p. 223.

(15) Eliel, J. C., *Am. J. Pharm.*, 69 (1897), 488.
(16) Scoville, W. L., *Drug. Circ.*, 43 (1899), 221.
(17) Wentworth, C. H., *Bull. Pharm.*, 13 (1899), 166.
(18) Haussmann, F. W., *Am. J. Pharm.*, 71 (1899), 102.
(19) Haussmann, F. W., *Ibid.*, 71 (1899), 121.
(20) Wells, E. H., *Ibid.*, 71 (1899), 254.
(21) Sieker, F., *Pharm. Rev.*, 20 (1902), 518.
(22) Raubenheimer, O., PROC. A. PH. A., 51 (1903), 384.
(23) "The Pharmacopœia of the United States of America," Eighth revision, 1905, p. 436.
(24) Francis, J. M., *Bull. Pharm.*, 20 (1906), 230.
(25) Cook, E. F., *Ibid.*, 22 (1908), 463.
(26) "The Pharmacopœia of the United States of America," Ninth revision, 1916, p. 427.
(27) Snyder, J. P., *Proc. Am. Drug. Manufacturers Assoc.*, 9 (1920), 251.
(28) Ewe, G. E., *Practical Drug.*, 40 (Nov., (1922), 24.
(29) Krantz, J. C., Jr., JOUR. A. PH. A., 12 (1923), 963.
(30) "The Pharmacopœia of the United States of America," Tenth revision, 1926, pp. 18, 370.
(31) Arny, H. V., Vener, B., and Jayne, L. C., JOUR. A. PH. A., 18 (1929), 384.
(32) Snyder, J. P., "Proceedings of the National Conference on Pharmaceutical Research," 1929–1930, p. 21.
(33) Ewe, G. E., *Ibid.*, 1929–1930, p. 21.
(34) Heyl, F. W., *Proc. Am. Drug. Manufacturers Assoc.*, 19 (1930), 264.
(35) Saalbach, L., "Proceedings of the National Conference on Pharmaceutical Research," 1929–1930, p. 22.
(36) Husa, W. J., JOUR. A. PH. A., 20 (1931), 759.
(37) Arny, H. V., Taub, A., and Steinberg, A., *Ibid.*, 20 (1931), 1153.
(38) Husa, W. J., and Klotz, L. J., *Ibid.*, 24 (1935), 45.

A Study of Isotonic Solutions*

By William J. Husa† and Oscar A. Rossi‡

There is an increasing interest in the use of medicinal solutions having the same osmotic pressure as the body fluids or bearing a definite relation thereto. Originally the main interest in this field was concerned with parenteral solutions, but recently considerable attention has been given to adjustment of the osmotic pressure of collyria and nasal preparations.

Hypotonic solutions may be made isotonic by addition of more of the drug or some other substance. The proportion of substance to be added may be determined experimentally or calculated mathematically. In the present paper, the value, limitations and accuracy of these methods are discussed and new experimental data are presented.

THEORETICAL

The idea of isotony is connected with the knowledge of osmotic pressure, which was first observed by Nollet in 1748. The term "osmosis" was introduced by Dutrochet.

Plasmolytic Method.—In 1888, De Vries found that when certain vegetable cells were placed in solutions containing 7.5% or more of sucrose, water passed out from the cells, which then contracted away from the sheath of cellulose. This phenomenon is called plasmolysis. By varying the concentration of the solution it is possible to determine the concentration at which plasmolysis ceases or is barely detectable; such solutions are said to be isotonic with the cell sap, *i. e.*, capable of producing the same pressure in the cell. The abnormal osmotic pressure of salt solutions was first observed by De Vries, who introduced the term "isotonic coefficient" to express the degree of deviation from normal. The plasmolytic method is not trustworthy for accurate measurements, as it is subject to numerous errors, such as reaction of the cell contents with the substances studied, exosmosis of cell contents, etc.

Hemolytic Method.—The hemolytic method, which was developed in 1890 by Hamburger, consists in the determination of the concentration which produces laking of red blood corpuscles, which are semipermeable to solutions of most substances except urea and ammonium salts. Wokes (1) determined the ratio *isotonic concentration/hemolytic concentration*, and found that this ratio was different for different substances. Some of the ratios were as follows: sodium chloride, 2; dextrose, 2.9; sodium bicarbonate, 3.1. Boric acid was hemolytic in all concentrations. Wokes concluded that if a solution diluted with half its volume of distilled water is hemolytic, it is not isotonic and should not be used for injection.

* Presented to the Subsection on Hospital Pharmacy of the A. PH. A., Detroit meeting, 1941.

† Head Professor of Pharmacy, University of Florida, Gainesville, Fla.

‡ Holder of a graduate scholarship of the National Commission of Culture of the Argentine Republic, 1940–1941.

Osmotic Pressure Laws.—The experimental methods available for the study of osmotic pressure were improved in 1866 by Traube, who showed that animal and vegetable cells could be replaced by an artificial membrane of copper ferrocyanide. Studies by Pfeffer and van't Hoff established the following laws: (*a*) the osmotic pressure of a solution is proportional to its concentration, and (*b*) osmotic pressure is proportional to absolute temperature. These laws may be represented by the expression (I) $P = K(T/v)$, where P = osmotic pressure, K = a constant, T = absolute temperature and v = volume of solution. It was suggested by van't Hoff, and later verified by others, that the value of the constant in the osmotic pressure equation would have the same numerical value as the constant in the gas equation. The general gas equation is (II): $p = (n/v)RT$, in which p = pressure, v = volume in liters, n = number of gram-molecules, R = gas constant and T = absolute temperature. The corresponding expression for osmotic pressure is as follows (III): $P = (n/v)RT = wRT/Mv = cRT$, where P = osmotic pressure, v = volume of the solution in liters, M = molecular weight of solute, w = number of Gm. of solute and c = moles of solute per liter.

From equation (III), which holds for very dilute solutions of nonelectrolytes, it follows that solutions of the same molar concentration are isosmotic, *i. e.*, have the same osmotic pressure, provided the temperature is the same. The term "isotonic" is commonly considered as synonymous with "isosmotic," but the distinction is sometimes made that the osmotic pressure of a solution depends on the total molar concentration, whereas the tonicity depends on the molar concentration of those solutes which do not pass through the particular plasmatic membrane (21).

In direct relation with the osmotic pressure of a solution is its vapor pressure, which is lowered in proportion to the concentration of dissolved substance. Direct measurements of osmotic pressure and vapor pressure are difficult to carry out. Hence it is customary to make use of two phenomena which are directly proportional to the lowering of the vapor pressure, *i. e.*, the elevation of the boiling point and the lowering of the freezing point. As the freezing-point determination offers several advantages, it is commonly employed.

In 1788, Blagden found that lowering of the freezing point is proportional to the concentration of solute. Raoult, in 1882, showed that the freezing-point lowering is proportional to the molar concentration of the solute and that, for a given solvent, equimolar solutions of different nonelectrolytes have the same freezing point. The mathematical expression of Raoult's law is as follows (IV): $\Delta = K(1000\ w/ML)$, where Δ = freezing-point lowering of the solution as compared with the pure solvent, K = molar freezing-point lowering of the solvent (this is an individual constant for each solvent, based on the freezing-point lowering given by 1 mole of solute in 1000 Gm. of solvent), w = weight of solute, M = molecular weight of solute and L = weight of solvent plus solute.

Raoult's law is valid only when the pure solvent crystallizes from the solution on freezing. Likewise the law is a limit law which holds only for dilute solutions. In higher concentrations, deviations occur as is the case with the gas laws at higher pressures.

For dilute solutions of nonelectrolytes, Raoult's law may be used in making calculations for the preparation of isotonic solutions, as shown in the examples (2) which follow.

Example 1: Calculate the quantity of anhydrous dextrose to be used in preparing 100 Gm. of a solution isotonic with blood serum.

Transforming equation (IV), we have (V):

$$w = \Delta ML/K \times 1000$$

It is known that the freezing-point lowering of blood is 0.56° C. and that the molar freezing-point lowering of water is 1.86° C. Substituting these figures for Δ and K, respectively, replacing M by 180 (the molecular weight of anhydrous dextrose), and replacing L by 100 (weight of solvent plus solute), we have: $w = 0.56 \times 180 \times 100/1.86 \times 100 = 5.42$ Gm. Hence the solution should be prepared by dissolving 5.42 Gm. of anhydrous dextrose in 94.58 Gm. of water.

Example 2: Calculate the quantity of anhydrous dextrose to be used in a 1% solution of urea to make 100 Gm. of solution isotonic with the blood.

Using equation (IV), the freezing-point lowering caused by 1% of urea is calculated as follows: $\Delta = 1.86 \times 1.0 \times 1000/60 \times 100 = 0.31°$.

The freezing-point lowering caused by the urea is subtracted from the freezing point of blood, to determine the additional lowering to be brought about by the addition of dextrose, as follows: $0.56° - 0.31° = 0.25°$. Using equation (V), and making the required substitution, we have: $w = 0.25 \times 180 \times 100/1.86 \times 1000 = 2.42$ Gm. The solution should thus be prepared by dissolving 1.00 Gm. of urea and 2.42 Gm. of anhydrous dextrose in sufficient water to make 100 Gm.

Raoult's law cannot be used to calculate the concentration of isotonic solutions of electrolytes, since solutions of acids, bases and salts show an abnormally high osmotic pressure. For such compounds the isotonic coefficient increases with dilution. These facts were explained by Arrhenius in his theory of electrolytic dissociation, which assumes that in solution the molecules of electrolyte dissociate into ions and each ion has an effect on the osmotic pressure. Laws of equilibrium between ions and undissociated molecules have been established for weak electrolytes, but with strong electrolytes, discrepancies are observed. In modern theories it is assumed that strong electrolytes are fully dissociated in solution. Consequently the effect of dilution on

osmotic pressure is explained on the basis of an increase in ionic activity, rather than by an increase in the number of ions as suggested by Arrhenius. It is thought that the ions, because of their electric charge, influence each other in their free movement and that these effects are greater in more concentrated solutions. Nixon and Culbert (3) have shown that the assumption of complete dissociation may be accepted for practical work in the case of monovalent electrolytes, but that in the case of sulfates of divalent cations the same assumption leads to considerable error.

THE PREPARATION OF ISOTONIC SOLUTIONS

Nonelectrolytes.—The use of Raoult's law in calculating the concentration of solutions isotonic with the blood has already been shown (see examples 1 and 2). Calculations for solutions isotonic with the tear secretion can be made in the same way, with the exception that in the formula 0.56 is replaced by 0.80, since the tear secretion freezes at −0.80° C.

Electrolytes.—The ordinary form of Raoult's law cannot be applied to electrolytes, because it does not take into consideration the electrolytic dissociation or ionic activity of the electrolyte. For use with electrolytes, Raoult's law must be modified by inclusion of the factor i, which is the value of the isotonic coefficient. Equation (V) thus becomes (VI): $w = \Delta ML/K \times 1000\,i$. The value of i cannot be predicted exactly because it depends on the nature of the compound and the degree of dilution. For 0.9% NaCl solution, the value of i has been determined experimentally and found to be 1.86. This value indicates that the osmotic pressure of a 0.9% NaCl solution is 1.86 times as great as that of an equimolar solution of a nonelectrolyte. When dealing with electrolytes, the practical value of Raoult's law is thus dependent on the accuracy with which the value of i is determined or estimated.

Osmotic Factor Method.—The osmotic pressure of various solutions may be compared by calculating an "osmotic factor" for each solution (4). The osmotic factor is proportional to the number of particles in 100 cc. of the solution and is calculated as follows (A):

$$\text{Osmotic factor} = \frac{\left(\begin{array}{c}\text{Number of particles}\\ \text{from one molecule of}\\ \text{solute}\end{array}\right)\left(\begin{array}{c}\text{Gm. of solute}\\ \text{in 100 cc. of}\\ \text{solution}\end{array}\right)}{\text{Gram-molecular weight of solute}}$$

The problems which present themselves usually take the following form. A solution of definite percentage strength is prescribed with directions that it be made isotonic with the blood. The osmotic factor of the blood cannot be calculated directly, but its value may be taken as equal to that of an isotonic solution of sodium chloride. The concentration of sodium chloride solution which is isotonic with the blood is still a subject of discussion and values ranging from 0.7% to 1.0% have been used. The experimental values found by several authors vary from 0.9% to 0.96%. The U. S. P. physiological solution of sodium chloride containing 0.85% NaCl is slightly hypotonic. Assuming that average blood is isotonic with 0.90% NaCl solution, the calculations may be made on this basis: osmotic factor (for 0.90% NaCl solution) = 2 × 0.9/58.5 = 0.031. The next step is to calculate the osmotic factor of the drug prescribed, using formula (A). The osmotic factor of the substance to be added is then determined as follows (B): osmotic factor of substance to be added = 0.031 − osmotic factor of drug prescribed.

The number of Gm. of substance to be added for 100 cc. of solution is calculated as follows (C):

$$\text{Number of Gm. of substance to be added for 100 cc.} = \frac{\left(\begin{array}{c}\text{Osmotic factor of}\\ \text{substance to be}\\ \text{added}\end{array}\right)\left(\begin{array}{c}\text{Gram-molecular}\\ \text{weight of substance}\\ \text{to be added}\end{array}\right)}{\left(\begin{array}{c}\text{Number of particles from one}\\ \text{molecule of substance to be added}\end{array}\right)}$$

For nonelectrolytes and feebly ionized substances such as boric acid the number of particles from one molecule is taken as 1. For strong electrolytes, complete dissociation is assumed and the number of particles is taken to be the same as the number of ions into which the compound dissociates.

Since complete dissociation is assumed for sodium chloride in formula (A), this compensates for part of the error incurred in assuming complete dissociation of strong electrolytes in formula (C). The error in assuming complete dissociation does not exceed 5%, provided the solution does not have a concentration greater than 0.1 molar (2). The method is thus satisfactory for practical work. One advantage of the method is that it is established on a weight/volume basis, which is more convenient for pharmacists than the weight/weight method of Raoult's law.

Example 3: Prepare 100 cc. of a 1% solution of cocaine hydrochloride using sufficient sodium chloride to make the solution isotonic with the blood.

Substituting in formula (A), we have: osmotic factor for cocaine hydrochloride = 2 × 1/339.7 = 0.006.

Using this result in formula (B), we get: osmotic factor of substance to be added = 0.031 − 0.006 = 0.025.

Substituting in formula (C): Number of Gm. of sodium chloride to be added in 100 cc. = 0.025 × 58.5/2 = 0.73. Therefore 0.73 Gm. of sodium chloride would be used in making 100 cc. of the solution.

In 1916, Zotier (24) described a method for calculating the concentration of isotonic solutions based on comparison with the number of dissolved particles (molecules or ions) contained in a solution of sodium chloride containing 9.5 Gm. of NaCl per L., the latter solution being considered isotonic with the blood. The isotonic coefficients of substances were taken from the early work of De Vries. By

formulating the expressions as a mathematical series for a variable number of substances, Zotier derived formulas which could be used to solve special types of problems involved in preparing isotonic solutions containing several different solutes, such as: (*a*) the molecular concentrations of the solutes shall be equal, (*b*) equal weights of the solutes shall be used, (*c*) the solutes shall be present in definite ratios as 3, 5, 7, etc. Zotier's work has the error that the calculations are made to the fourth decimal place (fourth significant figure), while the isotonic coefficients employed are not accurate to the first decimal place (second significant figure). The calculations thus give an illusion of extreme accuracy which is not justified, the real errors being more than a hundred times as great as would be inferred from the number of decimal places or significant figures employed in expressing the results.

Nicola's Method.—To calculate the weight of any substance required for making one liter of solution isotonic with the blood serum, which was taken to be isotonic with 0.95% NaCl solution, Nicola (8) used the following formula: $p = 0.302\ m/i$, where p = Gm. per liter, i = isotonic coefficient and m = molecular weight. The following approximate values are used for i: 1 for nonelectrolytes, 1.5 for electrolytes which give two ions, 2.0 for electrolytes which give three ions and 2.5 for electrolytes which give four ions. These values of i used by Nicola were based on the results obtained by De Vries in his early work on plasmolysis. Nicola's method is the same as that previously described by Zotier, except that Zotier used a value of 1.5 instead of 1.86 for the isotonic coefficient of NaCl.

Example 4: Calculate the concentration of an isotonic solution of sucrose. For sucrose, $m = 342$ and $i = 1$; therefore $p = 0.302 \times 342/1 = 103.28$ Gm. per liter.

Example 5: To render a 1% solution of cocaine hydrochloride ($m = 339.5$, $i = 1.5$) isotonic by addition of NaCl ($m = 58.5$, $i = 1.86$), Nicola calculates as follows: $10 \times 1.5/339.5 = 0.0441$ and $(0.302 - 0.0441)58.5/1.86 = 8.1$ Gm. per liter.

The result in example 5 is 8.1 Gm. NaCl per liter as compared with an experimentally determined (9) value of 7.4 Gm.

There is also an inconsistency in using a value of i for NaCl of 1.86 and a figure of 1.5 for other electrolytes which yield two ions.

For solutions isotonic with the tear secretion, Nicola's formula is: $p = 0.43\ m/i$.

Mellen and Seltzer (10) have used Nicola's method in compiling tables of sodium chloride tonicic equivalents of various drugs used in ophthalmic prescriptions. The use of these tables shortens the calculations. For convenience in dispensing, Mellen and Seltzer suggest that the pharmacist may keep on hand isotonic solutions of various drugs. Then in dispensing it will only be necessary to measure the quantity of isotonic solution of each drug prescribed representing the number of grains required and to add isotonic solution of sodium chloride to bring to the volume specified. However, there are some disadvantages in this procedure. Thus in some cases, *e. g.*, borax, an isotonic solution of the substance alone cannot be prepared, because the proportion required exceeds the solubility. Stock solutions of some of the drugs are impracticable because of deterioration.

Graphical Method.—Nixon (5) has described a graphical method intended to avoid the repetition of calculations for solutions of different percentage strengths. In the construction of the graph, two factors must be determined: (*a*) the *w/v* percentage of NaCl or other adjusting agent necessary to produce a solution isotonic with the blood serum or lachrymal secretion, and (*b*) the *w/v* percentage of the drug solution which is isotonic with the blood or tear secretion. Nixon calculated these values on the basis of the osmotic factor method, which was used in the form outlined by Brown (6). Value *a* is placed on the graph on one axis and value *b* is placed on the other axis. The fixed points are joined by a straight line. It is then possible to obtain, by direct reading from the graph, the percentage of adjusting agent which must be added to a given percentage solution of the drug in order to obtain an isotonic solution.

Fahmy (7) constructed similar graphs in which a number of drugs were included in each chart, the concentration of drug solution being plotted against the freezing-point lowering.

Freezing-Point Method.—The quantity of a substance required to make a given solution isotonic with the blood may be calculated by the method of Lumière and Chevrotier, as follows (11): $X = (0.56 - A)/B$, where X = number of Gm. of substance to be added for 100 cc. of the solution, A = freezing point of the unadjusted solution in degrees below 0° C., and B = freezing point of a 1% solution (*w/v*) of the substance to be added. This formula is included in the latest editions of the Swiss Pharmacopœia and British Pharmaceutical Codex.

The value of B has been determined experimentally for an umber of drugs. The value of A may be determined experimentally or it may be calculated by multiplying the strength of the solution expressed as percentage *w/v* by the value of B for the substance. In the latter calculation an error may arise, for example, if the freezing-point lowering of a 10% solution is not exactly ten times that of a 1% solution.

An analogous formula used in calculating the concentration of solutions isotonic with the tear secretion is: $X = (0.80 - A)/B$.

A table of the freezing-point lowering of various drugs and examples of calculations are given in the experimental part of the present paper.

Böhme's Method.—Böhme (2) has developed a method in which the freezing point of the drug solution is calculated, assuming complete dissociation of strong electrolytes, and the quantity of adjusting substance required to produce the remainder of the

freezing-point lowering is obtained by interpolation from a table of experimentally determined freezing points of solutions of various concentrations. The table includes a number of adjusting substances from which the pharmacist can make an appropriate selection. By using experimentally determined figures for the adjusting substance, the error caused by assuming complete dissociation is limited to the drug itself. For solutions not exceeding 0.1 molar concentration, the error has a maximum of 5%.

Modified Freezing-Point Method.—Another method (25) involves the use of a modification of Raoult's law, the value of i being taken from a table in which electrolytes are classified according to the number of ions and the nature of the negative ion.

EXPERIMENTAL

Experimental studies of isotonic solutions of a number of substances used in pharmacy have been

TABLE I.—FREEZING-POINT LOWERING OF 1% *W/V* SOLUTIONS IN °C.[a]

Alum (potassium)	$AlK(SO_4)_2.12H_2O$	0.11
Ammonium chloride	NH_4Cl	0.655
Antipyrine	$C_{11}H_{12}ON_2$	0.008
Atropine sulfate	$(C_{17}H_{23}O_3N)_2.H_2SO_4.H_2O$	0.08
Boric acid	H_3BO_3	0.315
Caffeine	$C_8H_{10}O_2N_4.H_2O$	0.075
Calcium chloride[b]	$CaCl_2$	0.42
Calcium gluconate	$C_{12}H_{22}O_{14}Ca.H_2O$	0.082
Calcium lactate	$Ca(C_3H_5O_3)_2.5H_2O$	0.12
Cocaine hydrochloride	$C_{17}H_{21}O_4N.HCl$	0.11
Cupric sulfate	$CuSO_4.5H_2O$	0.09
Dextrose	$C_6H_{12}O_6.H_2O$	0.095
Emetine hydrochloride	$C_{29}H_{40}O_4N_2.2HCl$	0.082
Ephedrine hydrochloride	$C_{10}H_{15}ON.HCl$	0.16
Ephedrine sulfate	$(C_{10}H_{15}ON)_2.H_2SO_4$	0.11
Ethylmorphine hydrochloride	$C_{19}H_{23}O_3N.HCl.2H_2O$	0.093
Glycerin	$C_3H_5(OH)_3$	0.205
Homatropine hydrobromide	$C_{16}H_{21}O_3N.HBr$	0.11
Lactose	$C_{12}H_{22}O_{11}.H_2O$	0.06
Magnesium chloride[b]	$MgCl_2.6H_2O$	0.245
Magnesium sulfate	$MgSO_4.7H_2O$	0.115
Mercuric chloride	$HgCl_2$	0.07
Mercuric cyanide[b]	$Hg(CN)_2$	0.08
Mercuric succinimide	$Hg(C_4H_4O_2N)_2$	0.08
Methenamine	$C_6H_{12}N_4$	0.14
Mild protein silver (Argyrol)		0.11
Morphine sulfate	$(C_{17}H_{19}O_3N)_2.H_2SO_4.5H_2O$	0.07
Phenol	C_6H_5OH	0.20
Physostigmine salicylate	$C_{15}H_{21}O_2N_3.HC_7H_5O_3$	0.11
Pilocarpine hydrochloride	$C_{11}H_{16}O_2N_2.HCl$	0.125
Potassium chloride[c]	KCl	0.485
Potassium iodide	KI	0.22
Potassium nitrate	KNO_3	0.35
Procaine hydrochloride	$C_{13}H_{20}O_2N_2.HCl$	0.14
Quinine hydrochloride[c]	$C_{20}H_{24}O_2N_2.HCl.2H_2O$	0.09
Quinine and urea hydrochloride	$C_{20}H_{24}O_2N_2.HCl.CO(NH_2)_2.HCl.5H_2O$	0.15
Silver nitrate	$AgNO_3$	0.225
Sodium benzoate	$NaC_7H_5O_2$	0.26
Sodium bicarbonate	$NaHCO_3$	0.40
Sodium biphosphate	$NaH_2PO_4.H_2O$	0.265
Sodium borate	$Na_2B_4O_7.10H_2O$	0.25
Sodium bromide	$NaBr$	0.36
Sodium cacodylate	$Na(CH_3)_2AsO_2.3H_2O$	0.175
Sodium chloride	$NaCl$	0.58
Sodium citrate	$Na_3C_6H_5O_7.2H_2O$	0.185
Sodium iodide	NaI	0.22
Sodium nitrate[d]	$NaNO_3$	0.36
Sodium salicylate	$NaC_7H_5O_3$	0.23
Sodium sulfate[d]	$Na_2SO_4.10H_2O$	0.165
Sodium thiosulfate	$Na_2S_2O_3.5H_2O$	0.178
Soluble iodophthalein	$C_{20}H_8O_4I_4Na_2.3H_2O$	0.07
Soluble phenobarbital	$C_{12}H_{11}O_3N_2Na$	0.155
Strong protein silver (Protargol)		0.02
Strong protein silver (Mallinckrodt's)		0.055
Sucrose	$C_{12}H_{22}O_{11}$	0.055
Tannic acid		0.02
Urea[d]	$CO(NH_2)_2$	0.31
Zinc sulfate	$ZnSO_4.7H_2O$	0.09

[a] Unless otherwise indicated, the substances used were of U. S. P. XI quality.
[b] Analytical reagent grade.
[c] N. F. VI quality.
[d] C. P. quality.

carried out by Poehl (12), Bennett and Gamble (13), Lumière and Chevrotier (11), Hattie (14), Van Itallie (9), Böhme (2), Nixon and Culbert (3), Whittier (15), Pinschmidt and Krantz (16), Picon (17), Dalimier (18) and Hirschlaff (19). Freezing-point data for solutions of some chemicals may also be found in the International Critical Tables, Smithsonian Tables and Landolt-Börnstein Tabellen. Such experimental data are frequently given in tables along with other less dependable data obtained by calculation. For this reason and because of the discrepancies in freezing points given in the literature by various authors, it was considered desirable to check the freezing-point determinations for a number of drugs, as well as to determine the freezing points of solutions of some drugs not previously recorded in the literature.

Apparatus.—The Beckmann freezing-point apparatus and thermometer were employed.

Materials.—In order that the results would be directly applicable to practical compounding, the drugs and chemicals used were of U. S. P. XI quality as a general rule, with a few exceptions in which the substances were of C. P. grade. In every case the material was taken from a previously unopened container in order to minimize the possibility of contamination or absorption of excess moisture.

Preparation of Solutions.—Solutions were prepared on a *w/v* basis, the substance usually being dissolved at room temperature and the solution brought to volume in a 100-cc. volumetric flask. If necessary, heat was used to hasten solution, and the liquid brought to 20° C. before adjusting to volume.

Procedure.—An approximate determination of the freezing point of each solution was first made, the solution being cooled rapidly by direct contact with the freezing mixture of ice, water and salt. A more accurate determination was then made by slowly cooling the solution, which was protected from direct contact with the freezing mixture by means of an air jacket. No correction was made for supercooling, since it was found convenient to avoid excessive supercooling by the addition of a small crystal of ice when the temperature had fallen a few tenths of a degree below the value obtained in the preliminary approximate test.

The experimental data obtained are given in Table I, the figure given for each substance being the average of at least three concordant determinations.

Examples of Calculations.—The use of the freezing-point method in preparing isotonic solutions is shown in the illustrations which follow.

Example 6: Prepare 100 cc. of a 3% solution of cocaine hydrochloride, using sufficient sodium chloride to make the solution isotonic with the blood.

Table I indicates that the freezing-point lowering of a 1% solution of cocaine hydrochloride is 0.11° C. Accordingly, the value of A (freezing-point lowering of a 3% solution of cocaine hydrochloride) is calculated as follows: $3 \times 0.11 = 0.33$. The value of B for sodium chloride as given in Table I is 0.58. The values of A and B are substituted in the formula. $X = (0.56 - A)/B = (0.56 - 0.33)/0.58 = 0.23/0.58 = 0.40$. Therefore 0.40 Gm. of sodium chloride would be used in making 100 cc. of the solution.

Example 7: Prepare 100 cc. of a 1% solution of silver nitrate, using sufficient sodium nitrate to make the solution isotonic with the tear secretion.

Table I indicates that the freezing-point lowering of a 1% solution of silver nitrate is 0.225 and that the value of B for sodium nitrate is 0.36. The values of A and B are substituted in the formula. $Y = (0.80 - A)/B = (0.80 - 0.225)/0.36 = 0.575/0.36 = 1.6$. The quantity of sodium nitrate to be used for 100 cc. of solution is 1.6 Gm.

Concentration of Isotonic Solutions.—On the basis of a survey of the literature, supplemented by experimental determinations in numerous instances, data were compiled regarding the percentage strength of isotonic solutions of various drugs. The results are presented in Table II. In using this table, it must be understood that these results are presented as numerical data, with no recommendations regarding possible medical use of the solutions. In some instances the solutions would not be suitable for medical use as such, *e. g.*, a 4.31% solution of silver nitrate would not be used in the eyes, but such solutions might be used in compounding.

TABLE II.—CONCENTRATION OF ISOTONIC SOLUTIONS[a]

Substance	Percentage Strength (w/v) of a Solution Isotonic With Blood (Δ = 0.56° C.)	Percentage Strength (w/v) of a Solution Isotonic With Tear Secretion (Δ = 0.80° C.)
Boric acid	1.8	2.4
Calcium gluconate	10.0	....
Calcium lactate	4.32[b]	....
Dextrose, anhydrous	5.1[b]	....
Lactose	9.5	....
Magnesium sulfate, crystalline	6.35[b]	....
Phenol	2.94[b]	....
Potassium chloride	1.12[b]	1.77[c]
Potassium nitrate	....	2.60[c]
Potassium sulfate	2.11[b]	....
Procaine hydrochloride	5.0	....
Silver nitrate	....	4.31[c]
Sodium benzoate	2.20	....
Sodium bicarbonate	1.4	2.0
Sodium borate	2.89[b]	4.10[c]
Sodium bromide	1.77[b]	....
Sodium cacodylate	3.3	....
Sodium chloride	0.95	1.40
Sodium citrate	3.0	....
Sodium iodide	2.5	3.58[c]
Sodium nitrate	1.4	2.05
Sodium phosphate, acid	2.64[b]	....
Sodium phosphate	4.53[b]	....
Sodium salicylate	2.4	....
Sodium sulfate ($Na_2SO_4.10H_2O$)	....	6.30[c]
Sucrose	10.0	....
Urea	1.8	....

[a] Unless otherwise indicated, the figures listed are experimental results of the present investigation.
[b] Figure taken from reference 13.
[c] Figure taken from reference 2.

DISCUSSION OF RESULTS

Freezing-Point Method.—The accuracy of the results obtained with the Beckmann apparatus is dependent on several factors. The rate of cooling is important, consequently care should be taken to obtain the same slow rate of cooling in all experiments. It is also necessary to avoid alterations due to absorption or evaporation of moisture, and to take care that the stirring takes place in the interior of the solution. Supercooling must be minimized or a correction made. Errors arise in the freezing-point method in case of some solutions in which the pure solvent does not separate on freezing. Difficulties are encountered in some instances in which the solubility of substances at 0° C. is much less than at room temperature.

An error may be introduced if the degree of dissociation or association at 0° C. is not the same as a body temperature. Other disadvantages of the freezing-point method are that data are available for only a limited number of substances and in some cases different values are given for the same substance in different tables (23).

Since Raoult's law is valid only for dilute solutions, an error arises in application of the law to solutions of higher concentration (22).

Some advantages of the freezing-point method are that it is suitable for all kinds of substances and is the only method which can be applied for substances of unknown molecular weight. An important advantage over methods of calculation is that the freezing-point method takes count of all changes in solution such as association, dissociation, common ion effect and other phenomena.

As previously stated, there is a chance for some error to be introduced in assuming that the freezing points of more concentrated solutions are proportional to the freezing point of a 1% solution.

TABLE III.—FREEZING-POINT LOWERING OF SOLUTIONS OF PROCAINE HYDROCHLORIDE

Concentration of Solution (w/v),%	Freezing-Point Lowering Calculated	Determined
1	....	0.14
2	0.28	0.255
5	0.70	0.54

As indicated in Table III, the results for procaine hydrochloride show that the freezing point of a 2% solution is not exactly twice that of a 1% solution and that the freezing point of a 5% solution is not five times that of a 1% solution. For drugs used in percentage strengths considerably removed from 1%, the accuracy would be improved by making experimental determinations of the freezing points of solutions of approximately the same concentration as that of the solutions used.

Form of Substance Used.—In all methods of determining the concentration of isotonic solutions, it is important to note whether the substance to be used is in the anhydrous form or in the form of a hydrate. Thus some authors have published data for zinc sulfate based on the anhydrous form, $ZnSO_4$, while others have used the same words "zinc sulfate" as applying to the official salt which is a hydrate, $ZnSO_4.7H_2O$. Needless to say, considerable errors may arise when the water of crystallization is not taken into consideration. To avoid these errors, the data in Table I in the present paper include the chemical formulas showing the exact number of molecules of water of crystallization in the samples used for the determinations.

The results in Table I indicate that the freezing points of 1% solutions of strong protein silver are different for different brands. Hence the freezing-point data obtained with one brand of protein silver cannot be applied to another brand.

Comparison of Methods.—It is of interest to compare the results obtained by different methods for the same solution.

Example 8: Prepare 100 cc. of a 1% solution of cocaine hydrochloride, using sufficient NaCl to make the solution isotonic with the blood. The results by various methods are as follows:

Method	Gm. NaCl
Experimental (freezing-point method)	0.74
Brown's formula	0.75
Osmotic factor method	0.73
Graphical method	0.75
Nicola's formula	0.81
Lumière and Chevrotier's formula	0.78
Böhme's method	0.77

The figures indicate that for a dilute solution of an alkaloidal salt, the results by a number of methods are fairly concordant for practical purposes. For salts giving two divalent ions and for more concentrated solutions of various compounds, the differences between the results obtained by various methods are greater.

In methods involving the use of the isotonic coefficient, a certain degree of error is always encountered in arriving at the value of i. The results on the value of i for the same substance are not exactly the same as determined by different methods, *e. g.*, freezing-point method, conductivity and direct determination of osmotic pressure (22). Application of mathematical formulas may lead to error where there are several solutes which may have mutual effects such as common ion effect, complex formation and changes in association or hydration (23).

In using the graphical method, a slight error arises from the fact that the graphs are drawn as straight lines, but actually the freezing-point depression may not be a linear function of the concentration (23). Furthermore the graphical method has the errors which are present in the data used in constructing the graphs.

Accuracy Required in Practical Work.—In considering isotonic solutions, it is common to take the freezing point of blood as −0.56° C. However, the freezing point of human blood in health may

vary between −0.49° C. and −0.59° C. (20). Because of this variation in natural blood, it is evident that extreme accuracy is not required in isotonic solutions for practical purposes.

For the tear secretion, most authors accept the freezing point as being −0.80° C. A few references give the freezing point of tears as −0.85° and −0.86° C., but practically all state that 1.4% solution of sodium chloride is isotonic with the tear secretion and such a solution of NaCl has a freezing point of −80° C.

General Rules.—A few general rules may be stated as follows:

Rule 1: Where the concentration of drug is 0.005 molar or less, the osmotic pressure of the drug may be neglected. In such cases a solution isotonic with the blood may be prepared by dissolving the drug in an isotonic vehicle such as physiological solution of sodium chloride, or a solution isotonic with the tear secretion may be made by dissolving the drug in a 1.4% solution of NaCl.

Rule 2: Where the concentration of drug is 0.1 molar or less, the calculation may be made by the osmotic factor method or freezing-point method or a combination of the two (Böhme's method). For strong electrolytes, full dissociation may be assumed in the osmotic factor method, the number of ions being deduced from the chemical formula of the compound.

Rule 3: Where the concentration of drug is greater than 0.1 molar, the freezing-point method is preferable.

Rule 4: Where the exact molecular weight of the drug is unknown, the freezing-point method may be used.

SUMMARY

The theoretical foundation for the preparation of isotonic solutions is outlined and methods for calculating the concentration of isotonic solutions are described. The advantages and limitations of the various methods are discussed.

New experimental data are given on the freezing points of solutions of a number of drugs. The application of the data in practical pharmaceutical work is illustrated by examples.

REFERENCES

(1) Wokes, F., *Quart. J. Pharm. Pharmacol.*, 9 (1936), 455.

(2) Böhme, H., *Arch. pharm.*, 274 (1936), 255.

(3) Nixon, W., and Culbert, R. C. A., *Pharm. J.*, 140 (1938), 411.

(4) Husa, W. J., "Pharmaceutical Dispensing," Second edition, Husa Brothers, Iowa City, Iowa, 1941, pp. 611–627.

(5) Nixon, W., *Pharm. J.*, 137 (1936), 568.

(6) Brown, H. T., *Ibid.*, 132 (1934), 633.

(7) Fahmy, I. R., *Rept. Pharm. Soc. Egypt*, 1 (1938), 26.

(8) Nicola, F., *Giorn. farm. chim.*, 70 (1921), 57.

(9) Van Itallie, E. I., *Pharm. Weekblad*, 55 (1918), 202; through YEAR BOOK, A. PH. A., 7 (1918), 100.

(10) Mellen, M., and Seltzer, L. A., JOUR. A. PH. A., 25 (1936), 759.

(11) Lumière A., and Chevrotier, J., *Bull. sci. pharmacol.*, 20 (1913), 711.

(12) Poehl, A., *Ibid.*, 1 (1899), 461.

(13) Bennett, R. R., and Gamble, F. W., *Pharm. J.*, 88 (1912), 354.

(14) Hattie, *Pharm. Weekblad*, 55, 202; through *Pharm. J.*, 102 (1919), 219.

(15) Whittier, E. O., *J. Phys. Chem.*, 41 (1937), 845.

(16) Pinschmidt, N., and Krantz, J. C., JOUR. A. PH. A., 29 (1940), 28.

(17) Picon, M., *J. pharm. chim.*, 12 (1930), 568.

(18) Dalimier, L., *Presse méd.*, 156 (1918), 372; through "Year Book Pharm. Trans. Brit. Pharm. Conf. London," 1918, p. 319.

(19) Hirschlaff, B., *Acta Med. Scand.*, 87 (1936), 530; through *Chem. Abstracts*, 30 (1936), 3000.

(20) Sollman, T., "A Manual of Pharmacology," Fifth edition, W. B. Saunders Company, Philadelphia, 1936, p. 776.

(21) Findlay, A., "Introduction to Physical Chemistry," Longmans, Green and Co., New York, 1933, p. 137.

(22) Bretau, P., *J. pharm. chim.* [7], 29 (1924), 544.

(23) Schorn, E. J., *Pharm. J.*, 142 (1939), 383.

(24) Zotier, V., *Bull. sci. pharmacol.*, 23 (1916), 219.

(25) Cook, E. F., and LaWall, C. H., "Remington's Practice of Pharmacy," Eighth edition, 1936.

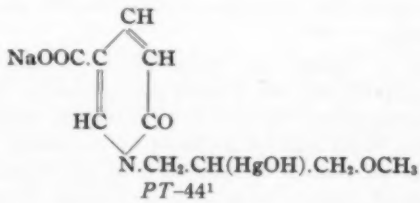
Organic Mercurials: The Influence of Structure on Absorption and Excretion*

By William H. Hunt, Lewis A. Walter and Russel J. Fosbinder

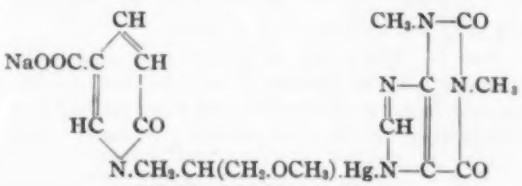
Clinical studies carried out by DeGraff and his co-workers (1) have demonstrated the marked increase in diuretic efficiency resulting from the addition of a xanthine, such as theophylline, to an organic mercurial. From pharmacological studies on Mercurpurin and Salyrgan-Theophylline, they concluded that the superiority of this type of combination was due to two factors, the increased rate and degree of absorption on intramuscular injection and the diminished local reaction occurring at the site of injection.

"Diuretic type" mercurials possess a common structure in that they consist of a cyclic structure containing a solubilizing group and a mercurated side chain joined to the nucleus through an amide linkage. Organic mercury compounds of this type appear to be regularly and rapidly absorbed from the site of injection with little or no retention of mercury by the body. It has been suggested that the absorption and possibly the excretion of these compounds is regulated to a considerable degree by the stability and relative position of the mercury–carbon linkage.

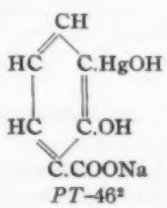
We considered it of interest to study the pharmacologic behavior of two organic mercurials, alone and in combination with theophylline or equivalent weakly acid nitrogen heterocyle. One compound was of the diuretic type, while the other contained mercury directly linked to the carbon atom in the aromatic ring structure. In our investigation, Salyrgan and Salyrgan-Theophylline were employed for purposes of comparison. From the chemical point of view each of the test compounds contained mercury in stable combination, the chief difference being in the position of the mercury–carbon bond. The chemical structure and nomenclature as well as the identification numbers of the compounds investigated are as follows:



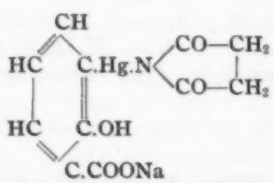
PT–44[1]



PT–44 with Theophylline



PT–46[2]



PT–46 with Succinimide

PT–44, a *N*-(mercurated propyl)-2-pyridone-5-carboxylic acid, was synthesized for study because it is structurally similar to existing diuretic type mercurials. The succinimide complex with *PT*–46 was employed because of the limited solubility of the corresponding theophylline compound.

EXPERIMENTAL

ABSORPTION FROM MUSCLE

In conducting the absorption studies we have made use of the technique described by DeGraff,

* From the Research Laboratories of the Maltbie Chemical Company, Newark, N. J.

[1] Sodium *N*-(β-hydroxymercuri-γ-methoxypropyl)-2-pyridone-5-carboxylate.

[2] Sodium 3-hydroxymercuri-2-hydroxybenzoate.

TABLE I.—ABSORPTION OF MERCURIALS FROM MUSCLE.

	Time, Hrs.	Time, Min.	Mercury Found, Mg.	Mercury Absorbed, Mean %
PT–44 with theophylline, 9.38 mg. of mercury injected		10	4.02	57.0
		20	3.46	63.1
		40	1.99	78.7
		60	...	...
		80	0.82	89.7
PT–44, 9.38 mg. of mercury injected	2		5.51	41.2
	4		2.80	69.5
	8		2.58	72.5
	16		1.74	81.5
	24		2.62	72.1
	48		1.78	81.2
PT–46 with succinimide, 11.64 mg. of mercury injected		10	5.72	50.8
		20	4.66	60.0
		40	3.92	66.3
		60	...	...
		80	2.11	81.8
PT–46, 11.64 mg. of mercury injected	2		6.98	40.1
	4		5.15	55.6
	8		4.91	57.8
	16		3.78	67.4
	24		3.36	71.1
	48		2.39	79.3
Salyrgan with theophylline,[a] 7.62 mg. of mercury injected		10	3.74	50.9
		20	2.61	67.0
		40	...	...
		60	0.12	96.6
		80	...	...
Salyrgan,[a] 7.62 mg. of mercury injected	2		5.20	31.8
	4		3.98	47.7
	8		2.95	61.3
	16		...	...
	24		1.20	84.3
	48		1.41	80.2

[a] Values obtained from a paper by DeGraff, *et al.* (2).

Batterman and Lehman (2), injecting measured quantities of the solutions into the tibialis anterior muscle of the rabbit. Mercury analyses were performed according to the procedure devised by Winkler (3) which is applicable for the determination of 0.05 to 0.5 mg. of mercury. A total of 71 normal male rabbits was used for this part of the investigation. Some of the values shown in Table I represent the average of 3 to 6 individual determinations. Values for the mean amount of mercury absorbed were obtained by subtracting that found in the muscle from the quantity injected initially.

The results obtained with *PT*–44, *PT*–46 and Salyrgan in combination with a weakly acidic nitrogen heterocyle are in substantial agreement with those reported by DeGraff, *et al.* (2), previously. Over 50% of the injected mercury was absorbed in the first 10 min.; thereafter the rate gradually decreased, but absorption was practically complete after 60 to 80 min.

PT–44, *PT*–46 and Salyrgan were absorbed at a greatly decreased rate, but it will be observed that the rate and degree of absorption did not vary significantly among the three compounds. Considering absorption data alone it would appear to be impossible to distinguish between a diuretic type and an ordinary organic mercurial, and one must conclude that in so far as rate of disappearance from tissue is concerned, the relative position of the mercury in the compound is without effect.

URINARY EXCRETION

Since absorption experiments failed to reveal any significant difference in behavior between the two types of compounds under investigation, it was decided to study the rate of urinary excretion in an attempt to rationalize previous views regarding chemical structure and diuretic action. In this connection Sollman, *et al.* (4), also DeGraff and co-workers (5), have shown clinically and experimentally that Salyrgan produces an intense and fairly immediate excretion of mercury following intramuscular injection, but the rate then declines rapidly due to a decrease in the rate of absorption. The total quantity of mercury voided in the urine of rabbits at varying time intervals following intravenous and intramuscular injection of the compounds was determined. For this purpose a total of 70 normal male rabbits was employed. Tables II and III show the results obtained by both routes of administration.

Referring to Table II, one observes the marked difference in the rate and amount of mercury excreted following the intramuscular injection of *PT*–46 with succinimide as compared to *PT*–44 and Salyrgan with and without theophylline. Less than 20% of *PT*–46 with succinimide was excreted in 72 hrs. While it is evident in the case of Salyrgan that theophylline significantly increased the rate of excretion of mercury, this effect was not so dramatic as the influence on the rate of absorption.

When *PT*–44 with theophylline and *PT*–46 with succinimide were injected intravenously a great difference in the rate of excretion was again noted. Table III shows that while over 50% of the former was excreted in 4 hrs., only 21% of the latter appeared in the urine after 24 hrs.

TABLE II.—EXCRETION OF MERCURY FOLLOWING INTRAMUSCULAR INJECTION

	Time, Hrs.	Mercury Excreted, Mg.	Mercury Excreted, Mean %
PT–44 with theophylline, 9.38 mg. of mercury injected	8	2.92	31.2
	16	3.45	36.9
	24	5.09	54.4
	48	...	...
	72	...	...
PT–44, 9.01 mg. of mercury injected	8	2.08	23.1
	16	3.77	41.9
	24	4.63	51.3
	48	5.85	64.9
	72	...	...
PT–46 with succinimide, 11.64 mg. of mercury injected	8	...	...
	16	...	...
	24	1.093	9.36
	48	...	...
	72	2.216	19.08
Salyrgan with theophylline, 7.92 mg. of mercury injected	8	4.543	57.4
	16	4.006	50.6
	24	5.02	63.38
	48	...	...
	72	...	...
Salyrgan, 7.92 mg. of mercury injected	8	1.49	18.85
	16	2.28	28.85
	24	3.523	44.43
	48	...	...
	72	...	...

TABLE III.—EXCRETION OF MERCURY FOLLOWING INTRAVENOUS INJECTION

	Time, Hrs.	Mercury Excreted, Mg.	Mercury Excreted, Mean %
PT–44 with theophylline, 9.38 mg. of mercury injected	1	2.703	28.81
	4	4.908	52.30
	7	4.962	52.94
	8	...	...
	24	6.828	72.84
	48	6.946	74.10
PT–46 with succinimide, 11.64 mg. of mercury injected	1	...	...
	4	1.77	15.20
	7	...	...
	8	2.62	22.50
	24	2.46	21.15
	48	...	...

TOXICITY

The intravenous toxicity of *PT*–44 with theophylline, *PT*–46 with succinimide and Salyrgan-Theophylline, also the intramuscular toxicity of *PT*–44 and Salyrgan with theophylline, were determined using a series of 92 male New Zealand white rabbits. The values for the acute toxicity, shown in Table IV, represent the equivalent weight of mercury per Kg. of body weight causing death in all animals within 30 min. following intravenous injection and death within 24 hrs. after intramuscular injection.

TABLE IV.—INTRAVENOUS AND INTRAMUSCULAR TOXICITY

Compound	Mg. of Mercury per Kg. of Body Weight: Intravenous	Mg. of Mercury per Kg. of Body Weight: Intramuscular
PT–44 with theophylline	10	60
PT–46 with succinimide	20	..
Salyrgan with theophylline	30	60

It is of interest to consider the difference in toxicity exhibited by *PT*–44 with theophylline and Salyrgan-Theophylline by the intravenous and intramuscular routes. While both compounds appear to be equally toxic by intramuscular administration, the pyridone derivative is three times more toxic than Salyrgan-Theophylline when injected intravenously. This observation was further confirmed by a series of toxicity determinations on the cat.

Although both derivatives are absorbed from muscle at rapid and almost identical rates, as shown previously, the amount of mercury in the blood does not reach the acute fatal level, even though five times the intravenous lethal dose of *PT*–44 with theophylline be administered by this route. Our observations on the relative toxicity of *PT*–44 and Salyrgan with theophylline led us to believe that the fatal effect of these compounds was primarily a direct action on the heart. To confirm this opinion, electrocardiograms from leads I and II were obtained before and after intravenous injection of maximum tolerated doses of each compound, using the rabbit as a test animal. The electrocardiograms showed both compounds exerted immediate but rather transitory toxic effects, characterized in the case of Salyrgan-Theophylline by left axis deviation, inversion of *T*-waves and marked increase in heart rate, while in the case of *PT*–44 with theophylline an exaggerated *T*-wave was apparent with but little change in rate.

DISCUSSION

A comparison of a new diuretic type mercurial, *PT*–44, with Salyrgan has confirmed the observations by DeGraff, *et al.* (2, 5), also Lehman and Dater (6), in respect to the effect of theophylline and related nitrogen heterocycles in enhancing the rate of absorption and excretion of mercury. An

organic mercurial such as *PT*–46, wherein the mercury is directly linked to a carbon atom of the cyclic nucleus, is not unlike a diuretic type compound in so far as absorption is concerned. When excretion studies are considered, the difference in behavior of the two types is marked. Nearly all of the injected mercury, in the case of *PT*–46, is retained and stored in the tissues. This difference cannot be ascribed to a variation in the stability of the mercury–carbon linkage but must be due to specific structural design which permits absorption from the circulation with subsequent metabolic destruction of the compound and retention of mercury.

Organic mercurial diuretics appear to exert a direct and pronounced toxic action on the heart when administered in sublethal and lethal doses. The toxic reaction produced by *PT*–44 with theophylline injected intravenously was much more intense than that caused by Salyrgan-Theophylline although both types of compounds possessed the same margin of safety on intramuscular administration.

SUMMARY

1. The relative position of the mercury–carbon linkage in an organic mercurial does not significantly influence the rate of absorption of the compound from muscle.

2. A weakly acid nitrogen heterocycle, theophylline or succinimide, enhances the rate of absorption of nondiuretic as well as diuretic types of compounds.

3. The type and position of mercury–carbon linkage exerts a profound influence on the retention of mercury whether it be administered intravenously or intramuscularly since a nondiuretic type is almost completely retained.

4. Succinimide coupled with a nondiuretic type of derivative does not significantly alter the degree of excretion of mercury.

5. Chemical stability of the mercury–carbon linkage appears to be of secondary importance in so far as absorption and excretion are concerned.

6. An organic mercurial derived from 2-pyridone-5-carboxylic acid was found to be more toxic than Salyrgan.

REFERENCES

(1) DeGraff, A. C., Nadler, J. E., and Batterman, R. C., *Am. J. Med. Sci.*, 191 (1936), 526.

(2) DeGraff, A. C., Batterman, R. C., and Lehman, R. A., *J. Pharmacol.*, 62 (1938), 26.

(3) Winkler, W. O., "Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists," Fifth edition, Washington, 1940, p. 409.

(4) Sollmann, T., Schreiber, N. E., Cole, H. N., DeWolf, H., and Ambler, J. V., *Arch. Dermatol. Syphilol.*, 31 (1935), 15.

(5) DeGraff, A. C., Batterman, R. C., Lehman, R. A., and Yasuna, E., *Proc. Soc. Exptl. Biol. Med.*, 39 (1938), 250.

(6) Lehman, R. A., and Dater, A., *J. Pharmacol.* 63 (1938), 443.

Bacteriostatic Properties of Certain Derivatives of Thiophene*

By H. W. Rhodehamel, Jr., and E. F. Degering

The chemical and physical properties of thiophene and its derivatives have been studied extensively by many investigators since its discovery by Victor Meyer in 1883 (1). No systematic study has been made, however, of the bacteriostatic properties of thiophene or its derivatives, although a few thiophene compounds have been used in medicine. Thiophene itself has been used as an antiseptic (2), sodium thiophenesulfonate and diiodothiophene have been used in the treatment of skin diseases (3), and tetrabromothiophene has been recommended as an efficient antiseptic (4). Many investigators believe that the large percentage of thiophene derivatives in ichthyol is responsible for its therapeutic properties (5, 6).

Recently a patent was issued in which it was claimed that aromatic mercury substituted derivatives of thiophene "have extraordinarily high potency as antiseptics and germicides and at the same time are characterized by relatively low toxicity and other desirable properties (7)." Several studies have shown that the replacement of a

* An abstract of portions of a thesis submitted to the Faculty of Purdue University, Lafayette, Ind., by H. W. Rhodehamel, Jr., in partial fulfillment of the requirements for the degree of Master of Science.

phenyl group by a 2-thienyl group does not alter, qualitatively at least, the pharmacological activity (8, 9, 10, 11).

With these and other facts in mind, it was thought it might be of value to synthesize certain derivatives of thiophene and to test them for bacteriostatic properties. No attempt has been made to produce a series of compounds to obtain comparative results within that series, but to produce instead miscellaneous derivatives which offer a means of comparison with compounds of known antiseptic value and which indicate future trends in research.

EXPERIMENTAL

A number of thiophene derivatives have been made by methods available in the literature. All syntheses started with thiophene obtained either from the Eastman Kodak Company or prepared from the fusion of sodium succinate and phosphorus trisulfide (12).

2-Acetothienone was prepared from thiophene by acetylating with acetyl chloride in the presence of fuming stannic chloride (13). This in turn was oxidized to 2-thiophenecarboxylic acid by hydrogen peroxide and potassium permanganate (14). The 2-thiophenecarboxylic acid was esterified to phenyl 2-thiophenecarboxylate by a modification of a method presented in "Organic Syntheses" (15). 5-Chloromercuri-2-thiophenecarboxylic acid was prepared by the interreaction of 2-thiophenecarboxylic acid and mercuric chloride in the presence of mercuric acetate (16).

The reaction between mercuric acetate and 2-thiophenecarboxylic acid yielded a product whose properties did not correspond to those of the predicted product, 5-acetoxymercuri-2-thiophenecarboxylic acid. The compound obtained is assumed to be the mercuric acetate salt of 2-thiophenecarboxylic acid.

2-Chloromercurithiophene was prepared by the reaction between thiophene and mercuric chloride in the presence of sodium acetate in the cold (17).

2,5-Dichloromercurithiophene was obtained by refluxing thiophene with mercuric chloride in the presence of sodium acetate (18).

2-Chloromercuri-5-chlorothiophene was prepared from 2-chlorothiophene which was made by the method of Töhl and Eberhard (19) and mercuric chloride in the presence of sodium acetate (20).

2,5-Bis(acetoxymercuri)-thiophene was prepared from thiophene and mercuric acetate in the cold (21). 2,3-Bis(acetoxymercuri)-5-nitrothiophene was obtained by refluxing 2-nitrothiophene (22) and mercuric oxide in glacial acetic acid for two hours (23).

Diphenyl-2-thienylacetic acid was prepared by the condensation of benzilic acid with thiophene in glacial acetic acid and concentrated sulfuric acid (24). Triphenylacetic acid was prepared from benzilic acid and benzene in the presence of fuming stannic chloride (25).

Phenyl benzoate was prepared by a modification of a method presented in "Organic Syntheses" (15).

Testing of the Compounds.[1]—Because of the range of solubilities of these compounds and the attending difficulty of obtaining solvents for each, preliminary bacteriostatic testing has been confined to a spot test. In these tests the standard *Staphylococcus aureus* strain No. 209 was used in poured agar plates made in the usual way, both with plain agar and with a 10% serum agar. The undiluted compound was applied like patch tests to both the plain and serum agar. Following incubation at 37° C. for 24 hrs., the zones of clearing about the applied compound were measured. The dimensions of the zone of clearing are indicative of the antiseptic property of the compound being tested, the relationship being

[1] The bacteriostatic testing was carried out by the Lilly Research Laboratories, Indianapolis, Ind.

TABLE I.—BACTERIOSTATIC TESTING

	Compound Tested	Zone of Clearing, Mm., on *Staph. aureus* Poured Agar Plates Using Plain Agar	10% Serum Agar
(1*a*)	Acetophenone	30	10
(1*b*)	2-Acetothienone	20	20
(2*a*)	Benzoic acid	10	Precipitation
(2*b*)	2-Thiophenecarboxylic acid	8	Precipitation
(3*a*)	Phenyl benzoate	1	1
(3*b*)	Phenyl 2-thiophenecarboxylate	5	2
(4*a*)	Triphenylacetic acid	1	1
(4*b*)	Diphenyl-2-thienylacetic acid	9	5
(5*a*)	Phenyl mercuric chloride	10	10
(5*b*)	2-Chloromercurithiophene	10	10
(5*c*)	Pyridyl mercuric chloride	30	12
(6)	2,5-Bis(acetoxymercuri)-thiophene	10	5
(7)	2,3-Bis(acetoxymercuri)-5-nitrothiophene	7	7
(8)	5-Chloromercuri-2-chlorothiophene	7	5
(9)	5-Chloromercuri-2-thiophenecarboxylic acid	10	10
(10)	2,5-Dichloromercurithiophene	7	5
(11)	Product of the reaction between mercuric acetate and 2-thiophenecarboxylic acid	15	16
(12)	Merthiolate (sodium ethyl mercuri thiosalicylate, Lilly)	30	15

directly proportional (*cf.* Circular 198 of the Department of Agriculture (26)).

Interpretations of the Bacteriostatic Testing.—The results of the bacteriostatic testing of certain derivatives of thiophene and certain isosteric compounds are given in Table I. Merthiolate (sodium ethyl mercuri thiosalicylate, Lilly) has also been tested and included as an antiseptic of known properties.

Although it is inadvisable to draw conclusions from too few data, it appears from these tests that the substitution of the thiophene nucleus for a phenyl group either does not change the bacteriostatic properties or renders the derivative somewhat more active. For example, diphenyl-2-thienylacetic acid is nine times more effective against *Staphylococcus aureus* in plain agar than is triphenylacetic acid and five times as effective in serum agar. Similarly, phenyl 2-thiophenecarboxylate exhibits greater activity than phenyl benzoate.

Phenyl mercuric chloride and 2-thienyl mercuric chloride are of about the same activity. Acetophenone and 2-acetothienone are of the same order of activity; one is more active in plain agar, the other in serum agar. These two compounds are both liquids and cannot accurately be compared to the solid derivatives.

2-Thiophenecarboxylic acid demonstrates rather low activity, yet it compares favorably with benzoic acid. Tests could not be carried out for either of these compounds in serum agar since they both cause precipitation of the serum. The other derivatives showed no outstanding activity.

SUMMARY

1. Eleven derivatives of thiophene were prepared and their bacteriostatic properties tested along with closely related compounds of the benzene series and certain other substances against *Staphylococcus aureus* both in plain agar and in 10% serum agar.

2. No compound showed outstanding bacteriostatic activity.

3. The thiophene nucleus substituted in a compound for the phenyl group seems to cause in the derivative either the same or a somewhat enhanced activity against *Staphylococcus aureus* in comparison with the original substance.

REFERENCES

(1) Meyer, *Ber.*, 16 (1883), 1465.

(2) Fuller, "Chemistry and Analysis of Drugs and Medicines," 1920, p. 747.

(3) Wood and LaWall, "U. S. Dispensatory," Twenty-second edition, 1937, p. 1615.

(4) Fuller, "Chemistry and Analysis of Drugs and Medicines," 1920, p. 748.

(5) U. S. Bureau of Mines, Circular No. 7042 (1938).

(6) Leclère, *Compt. rend.*, 194 (1932), 286.

(7) Andersen, U. S. Patent 2,085,065 (Feb. 25, 1935).

(8) Steinkopf and Ohse, *Ann.*, 437 (1924), 14.

(9) Ghosh, *J. Indian Chem. Soc.*, 14 (1937), 713; through *Chem. Abstracts*, 32 (1938), 4166.

(10) Blicke and Zienty, *J. Am. Chem. Soc.*, 63 (1941), 2945.

(11) Blicke and Burckhalter, *Ibid.*, 64 (1942), 477.

(12) Whitmore, "Organic Syntheses," 12 (1932), 72; Friedburg, *J. Am. Chem. Soc.*, 12 (1890), 83.

(13) Fuson, "Organic Syntheses," 18 (1938), 1.

(14) Voerman, *Rec. trav. chim.*, 26 (1906), 293.

(15) Allen, "Organic Syntheses," 20 (1940), 77.

(16) Steinkopf, *Ann.*, 413 (1916), 319.

(17) Volhard, *Ibid.*, 267 (1892), 176.

(18) Steinkopf and Killingstad, *Ibid.*, 532 (1937), 290.

(19) Töhl and Eberhard, *Ber.*, 26 (1893), 2947.

(20) Steinkopf and Bauermeister, *Ann.*, 403 (1914), 66.

(21) Steinkopf and Killingstad, *Ibid.*, 532 (1937), 292.

(22) Hartmann, "Organic Syntheses," 14 (1934), 76.

(23) Steinkopf, *Ann.*, 545 (1940), 42.

(24) Ancizar-Sordo and Bistrzycki, *Helv. Chim. Acta*, 14 (1931), 141.

(25) Bistrzycki and Mauron, *Ber.*, 40 (1907), 4062.

(26) Circular No. 198, U. S. Department of Agriculture, 1931, p. 10.

A Study of Athlete's Foot and Its Control, II*

By R. E. Williamson† and H. G. DeKay‡

A vast amount of research has been carried on during the past quarter of a century, with a view toward finding a fungicide or a method capable of controlling dermatomycosis or dermatophytosis. These terms are commonly used interchangeably with epidermophytosis, epidermomycosis, trichophytosis, "ringworm" and "athlete's foot," all denoting any superficial fungus infection of the glabrous or intertriginous areas of skin.

It has been variously estimated that from 50% to 90% of the population of the United States are affected at some time during their lives, and Gilman (1) noted that of 390 new patients with diseases of the skin observed during a six months' period in the Student Health Service of the University of Pennsylvania, 145, or 37%, had a dermatophytic infection.

Legge, Bonar and Templeton (2) found that 53.3% of the men and 15.3% of the women of the 3100 freshmen in the University of California were infected, and at the end of the spring semester, 78.6% of the men and 17.3% of the women had a mycotic infection of the feet.

Prehn (3) found that 88% of 1500 men examined on eleven ships of the Navy in various parts of the world showed clinical evidence of a fungus infection of the feet.

Among the various chemicals recommended for the prevention and treatment of these fungus infections of the feet is sodium hypochlorite.

Gilman (1), Hadfield (5), and Vaughan and DeKay (6) all agree with Osborne and Hitchcock (4) that the fungus infection problem can be solved by control of exposure in swimming pools, showers, gymnasiums, etc., with foot baths containing 0.5% to 1.0% sodium hypochlorite solution.

Another series of workers, including Shaffer (7), Weirich (8), and Chiles (9) seem to disagree with the above-mentioned workers.

No species of fungi or technique has been generally accepted as a basis for comparative fungicidal tests. As a result, the literature on this subject is filled with apparent inconsistencies as to the comparative fungicidal powers of certain commonly used chemicals as well as methods for determining the fungicidal properties of these chemicals. The primary aim of this work was to continue the investigation started in 1940 (7) on the use of sodium hypochlorite solution.

EXPERIMENTAL

In this study five fungi, characteristic examples of the three main genera of Dermatophytes, were used. These five were chosen because of their common occurrence in the majority of cases of dermatophytosis. They were as follows: (1) *Trichophyton rosaceum*, (2) *Trichophyton interdigitale* 7190, (3) *Microsporon lanosum* 650, (4) *Achorion schoenleinii* 641 and 642, and (5) *Trichophyton gypseum asteroides* 7189. These five fungi were all grown successfully on Sabouraud's *A* culture medium in 8-ounce prescription bottles, and allowed to incubate at room temperature for three to four weeks to obtain maximum sporulation. A large quantity of inoculum was prepared at one time, consisting of a standardized number of spores (10,000/cc.) and finely comminuted mycelium suspended in normal saline solution almost completely freed from nutrient materials.

In testing, two 4-mm. loopfuls of the inoculum were placed in 5 ml. of the chemical and after proper time exposure, two 4-mm. loopfuls of this mixture were removed and introduced into 5 ml. of broth subculture. Slight amounts of the chemical being tested were carried over into the broth tubes, and in order to balance this, two loopfuls of the fungicide were added to each control tube in which the untreated spores were planted.

Samples of sodium hypochlorite foot baths were collected at the Purdue University field house, analyzed for their sodium hypochlorite content, and these samples tested for their relative fungicidal value using the five fungi heretofore mentioned.

* An abstract of a thesis presented to the faculty of Purdue University by R. E. Williamson, in partial fulfillment of the requirements for the degree of Master of Science.

† Graduate Assistant in Pharmacy, Purdue University, Lafayette, Ind.

‡ Associate Professor of Pharmacy, Purdue University.

Samples analyzed at 0.215, 0.290, 0.508 and 0.701% of sodium hypochlorite were tested in this manner. In each case the inoculi were exposed to the sample for 10-, 20-, 30-, and 60-sec. intervals, and the subcultures observed for growth every 24 hrs. for twenty days. Table I shows the results.

TABLE I

NaOCl, %	0.215				0.290				0.508				0.701			
Time of exposure, min.	10	20	30	60	10	20	30	60	10	20	30	60	10	20	30	60
Fungus 1	+	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−
2	+	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−
3	+	+	−	−	+	+	+	−	−	−	−	−	−	−	−	−
4	+	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−
5	+	+	−	−	+	+	−	−	−	−	−	−	−	−	−	−
Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE II

NaOCl, %	0.1			0.2			0.3			0.4			0.5			0.6		
Time of exposure, min.	10	30	60	10	30	60	10	30	60	10	30	60	10	30	60	10	30	60
Fungus 1	+	+	−	+	+	−	+	−	−	+	−	−	−	−	−	−	−	−
2	+	+	−	+	+	−	+	−	−	+	−	−	−	−	−	−	−	−
3	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

TABLE III

NaOCl, %	0.1			0.2			0.3			0.4			0.5			0.6		
Time of exposure, min.	10	30	60	10	30	60	10	30	60	10	30	60	10	30	60	10	30	60
Fungus 1	+	+	−	+	+	−	+	−	−	+	−	−	−	−	−	−	−	−
2	+	+	−	+	+	−	+	+	−	+	−	−	+	−	−	−	−	−
3	+	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
4	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
5	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−

TABLE IV

NaOCl, %	0.1			0.2			0.3			0.4			0.5			0.6		
Time of exposure, min.	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Fungus 1	+	+	−	+	+	−	+	−	−	+	−	−	−	−	−	−	−	−
2	+	+	−	+	+	−	+	+	−	+	+	−	+	−	−	−	−	−
3	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	−	−	−															
5	−	−	−															

TABLE V

NaOCl, %	0.1			0.2			0.3			0.4			0.5			0.6		
Time of exposure, min.	10	30	60	10	30	60	10	30	60	10	30	60	10	30	60	10	30	60
Fungus 1	+	+	−	+	+	−	+	+	−	+	−	−	+	−	−	−	−	−
2	+	+	−	+	+	−	+	+	−	+	+	−	+	−	−	−	−	−
3	+	+	−	+	+	−	−	−	−									
4	+	−	−	−	−	−												
5	+	−	−	−	−	−												

The test for the efficiency of any fungicide is its ability to destroy or prevent the growth of organisms. The most reliable procedure would be to add definite amounts of fungi to the solutions being tested in order to be certain of the activity of the fungicide. In the second part of this study, foot-bath solutions of 500-ml. volume were prepared containing sodium hypochlorite in concentrations ranging from 0.2% to 0.6%.

Specific amounts of inoculi (1 ml. and 5 ml.) were added to these foot-bath solutions. The larger amount would contain more of the organism than a foot bath which had been in use for 24 hrs.

The baths were vigorously stirred from the time the inoculum was added until the end of the test. Two 4-mm. loopfuls of the solution were removed after 10, 30, and 60 seconds' exposure to the chemical, and placed in subcultures of beef broth, with the following results. Table II shows the results after adding 1 ml. of inoculum to each bath while Table III shows the results after adding 5 ml. of inoculum to each bath.

The third part of this experimentation was to determine the activity of the fungicide on foot baths containing definite percentages of sodium hypochlorite and known amounts of inoculum together with organic matter in the form of hide powder, thereby more nearly approaching the conditions of the foot bath which had been in use.

Foot baths were prepared containing sodium hypochlorite ranging from 0.1% to 0.6%. These baths also contained 1 ml. and 5 ml. of inoculum together with 0.5 Gm. of hide powder to each 500 cc. of solution. Table IV gives the results for 1 ml. of inoculum and Table V gives the results for 5 ml. of inoculum.

A study of Tables II to V, inclusive, shows that the amount of inoculum and the presence of organic matter do have a definite effect upon the activity of the fungicide.

SUMMARY

Foot baths containing 0.3% of sodium hypochlorite will prevent the growth of *Trichophyton rosaceum, Trichophyton interdigitale, Microsporon lanosum, Achorion shoenleinii* and *Trichophyton gypseum asteroides* after 30 seconds' exposure and baths containing 0.5% of sodium hypochlorite will prevent the growth of these fungi after 10 seconds' exposure.

REFERENCES

(1) Gilman, R. L., *J. Am. Med. Assoc.*, 100 (1933), 715.

(2) Legge, F. T., Bonar, L., and Templeton, R. F., *Ibid.*, 93 (1929), 170.

(3) Prehn, D. T., *Ibid.*, 111 (1939), 685.

(4) Osborne, E. D., and Hitchcock, B. S., *Ibid.*, 97 (1931), 453.

(5) Hadfield, W. A., *Soap*, 11 (1935), 121.

(6) Vaughan, J. B., and DeKay, H. G., JOUR. A. PH. A., 29 (1940), 260.

(7) Shaffer, L. W., and Cary, W. H., *J. Mich. State Med. Soc.*, 32 (1933), 648.

(8) Weirich, C. L., *Soap*, 16 (1940), 88.

(9) Chiles, H. M., *Ibid.*, 16 (1940), 111.

Book Reviews

Chemical Analysis. Volume II, Chromatographic Adsorption Analysis, by HAROLD H. STRAIN, Ph.D. Interscience Publishers, Inc., 215 Fourth Avenue, New York, 1941. x + 222 pp., 37 figs., 15 x 22.5 cm. Price, $3.75.

The aim of this book according to the preface, is to present a summary of the present knowledge concerning applications of the chromatographic adsorption method of analysis. Experimental procedures and applications of the method to problems involving the detection and isolation of natural products in a pure form are especially emphasized. Fundamental theories are also stressed. Different types of apparatus used in chromatographic adsorption methods are adequately described and clearly illustrated by means of diagrams, so that they may be readily assembled from apparatus at hand in any well-equipped laboratory. Information on adsorbents, solvents and eluants is unusually concise, but at the same time complete enough to serve as a guide to the research worker who is using the method for the first time.

The section on the chromatography of organic compounds is particularly interesting. It should serve as a stimulus to pharmaceutical chemists working with plant or animal material to attempt the application of chromatic adsorption methods where more conventional procedures have failed. A few of the topics covered are the applications of the method to the separation and isolation of hydrocarbons, acids, fats, amino acids, carbohydrates, terpenes, benzene derivatives, sterols and steroids, heterocyclic nitrogenous bases, vitamins, hormones, enzymes and plant pigments.

The book is excellently written and well documented throughout. The bibliography is quite extensive, but no claim to completeness is made by the

author because many of the important foreign scientific journals have not been available since 1939. The utility of the book is further enhanced by good author and subject indexes.

The volume should be interesting to any pharmaceutical chemist, and indispensable to any investigator whose work involves the isolation of organic plant principles.—JUSTIN L. POWERS.

Minerals in Nutrition, by ZOLTON T. WIRTSCHAFTER, M.D. Reinhold Publishing Corporation, 330 West Forty-second Street, New York, 1942. 175 pp., 8 illus., 12.5 x 18.5 cm. Price, $1.75.

Minerals in Nutrition is intended to be a popular treatise comparable to similar books on the vitamins. While many interesting facts are told, often the language is too technical for the layman. Admittedly, the minerals are not so dramatic as the vitamins, yet it is believed that the story of the important function of these elements in human physiology could be told in a more vivid manner. Certainly the discovery of the role of iodine in endemic goiter is on a par with that of nicotinic acid in pellagra.

The more serious-minded student of nutrition will be interested in the many tables and the references to more extended discussions.—M. W. GREEN.

Chemistry and Physiology of the Vitamins, by H. R. ROSENBERG, Sc.D. Interscience Publishers, Inc., 215 Fourth Avenue, New York, 1942. xix + 674 pp., 15 x 23 cm. Price, $12.00.

There are several books available on special topics relating to vitamins, and monographs on single vitamins, but in this volume there is presented a comprehensive treatment of the chemistry and physiology of all the known vitamins. According to the preface, an attempt has been made to cover all topics of vitamin research and especially the chemistry and physiology of the vitamins. This volume is presented with the hope that it may guide the student and scholar through our present-day knowledge of the field, and inspire further development. Both of these aims of the author should be more than realized by this excellent treatise on the vitamins.

The text of the volume is divided into sixteen chapters and an appendix. For the want of a better classification, the vitamins are presented in alphabetical order of nomenclature. The book begins with a chapter devoted to a general discussion of the vitamins in which this class of compounds is defined and sharply differentiated from the hormones and from other essential and nonessential food constituents.

In general, the same arrangement of subject matter relating to individual vitamins is followed in each succeeding chapter. The arrangement of the chapter on vitamin B_1—thiamin—may be used as an example of the general arrangement of the chapters. The first section, devoted to nomenclature and survey, lists the names which have been applied to thiamin and includes the chemical name, the structure, the empirical formula and the number of international units of vitamin B_1 per gram. The succeeding sections discuss in order the chronology, occurrence, isolation, properties, chemical constitution and synthesis, industrial methods of preparation, biogenesis, thiochrome, vitamin B_1-pyrophosphate, specificity of vitamin B_1 action, methods of determination, standards, physiology of plants and microorganisms, animal physiology, avitaminosis and hypovitaminosis, hypervitaminosis, and requirements for thiamin. This chapter includes 339 literature citations and is representative of the completeness of documentation throughout the book.

The appendix includes a brief chapter on the "vitagens," in which the essential fatty acids, amino acids, carbohydrates and organic sulfur-containing compounds are discussed briefly. Another valuable feature of the appendix is a list and abstracts of vitamin patents which have been issued in the United States, Great Britain, France and Germany.

From a glance at the physiological sections of this book, it is evident that the author is a chemist and not a physiologist. These sections do not suffer from a lack of completeness, but rather from a failure to evaluate the information. This may be seen, for example, in the case of ascorbic acid, where the alleged carrier role of the ascorbate is overly emphasized and the obscure actions of this vitamin on many enzyme systems are catalogued with no coordinated viewpoint. However, since there are many other monographs available which present the vitamins from the physiological and medical viewpoint, this is not a particularly grievous fault and the quality of the other portions of the book well compensate for it.

While this volume represents a scholarly presentation of all information relating to the vitamins which will appeal most strongly to teachers and investigators in the vitamin field and related fields, it should also prove of inestimable value to the pharmacist. It should appeal to the pharmacist because the vitamins constitute a class of products more widely distributed by pharmacists than any other single group of substances. As the final distributor, the pharmacist must be informed upon all phases of the chemistry and physiology of vitamins so that he may advise the ultimate consumer intelligently. In this book will be found the answer to practically any conceivable question relating to vitamins for which an answer exists.—JUSTIN L. POWERS.

The Rat in Laboratory Investigation, edited by JOHN Q. GRIFFITH, JR., and EDMOND J. FARRIS. J. B. Lippincott Company, Philadelphia, 1942. xiv + 488 pp., 178 illus., 15 x 23 cm. Price, $7.50.

The Rat in Laboratory Investigation is a "must" book for every person doing animal experimentation involving the use of rats. This book is a compilation of the practical experiences of 30 different

authors and serves as a useful handbook and laboratory guide. The following major subjects are discussed: breeding of the rat, general methods for handling, gross anatomy, experimental methods and rat embryos, dietary requirements of the rat, the teeth, the digestive system, metabolism, the central nervous system, techniques for the investigation of psychological phenomena, the circulatory system, the use of the rat in the bioassay of hormones, dosage of drugs for rats, hematology of the rat, radiologic considerations, surgery of the rat, histologic methods adapted to rat tissues, the osseous system, the eye of the albino rat, protozoan parasites of the rat, metazoan parasites of the rat, and spontaneous diseases of the rat. Here is to be found the essential information for every worker in the biological sciences with many references to more extended information in each of the fields represented.

Space will permit a review of but a few of the subjects covered by this excellent book. The chapters on the breeding of the rat, general methods of handling and the spontaneous diseases of rats will be found very helpful in a general way to any animal laboratory. These chapters contain such information as the time of gestation, preparation of the nest, size of litters, methods of caging, age determination, identification of individual animals, convenient ways of keeping vital records, ways of keeping the animals free from disease, methods of producing anesthesia, and the like. Even workers of long experience will find helpful tips in these sections.

Researchers in the field of nutrition will be grateful for the chapter on dietary requirements. Methods are given for the production of mineral deficiencies, vitamin deficiencies and amino acid deficiencies. Especially useful is a summary of known requirements for the rat of each of the vitamins and minerals (p. 97). The chapter on metabolism is also helpful to anyone wishing to study the effects of variants upon metabolism.

To pharmacy researchers, the most useful chapter is that on the "Dosage of Drugs for Rats," by Harald G. O. Holck. The factors that modify dosage of drugs are discussed and in this connection it is particularly gratifying to the reviewer to note the emphasis placed upon diet. In making adequate studies of drug action and particularly assay, the control of the diet is believed to be very important. Since the action of certain metals such as potassium and calcium is so profound and the need for some of the vitamins for proper nerve function is so well established, it is amazing how little attention is often paid to the state of nutrition of experimental animals. The most useful section of this chapter is the extensive tabulation of dosage of drugs covering 35 pages and giving references to the original literature. Information of this type is used time and time again by workers in the field, but is often found only with considerable difficulty. In usefulness this chapter alone is well worth the price of the book.

The compilation of a handbook of this sort, involving so many variable viewpoints, is extremely difficult. The relative lack of overlapping of material and the unity of style is a tribute to fine editorial craftsmanship—M. W. GREEN.

Preparacion de Productos Quimicos y Quimico-Farmaceuticos by C. A. ROJAHN, Director of the School of Pharmacy of the University of Halle. Trans. from the original German and amplified by FRANCISCO GIRAL, Head of the Department of Organic Synthesis in the Hormona Laboratories, Mexico. Editorial Atlante, S. A., Mexico, D. F., 1942. xxxix + 1002 pp., 17 x 23 cm.

These two volumes are intended to be used as references for the preparation of medicinal chemicals and their intermediates. Many other pertinent data, such as yields, physical properties, tests for identity and purity (largely taken from the German Pharmacopœia) and a bibliography, are also provided.

The volumes are divided into two parts. Part I is a study of inorganic substances, the elements being classified similarly to the Periodic Table. Part II, consisting of approximately 700 pages, deals with the organic compounds, classified in the traditional manner. Here may be found such modern therapeutic agents as thiamine, nicotinic acid, pentamethylenetetrazol, and others. One of the unique features of this section is a schematic diagram of the synthesis of the organic arsenicals.

To anyone for whom the language (Spanish) is no handicap, these volumes will be a valuable addition to the reference library.—M. W. GREEN.

JUST OUT...

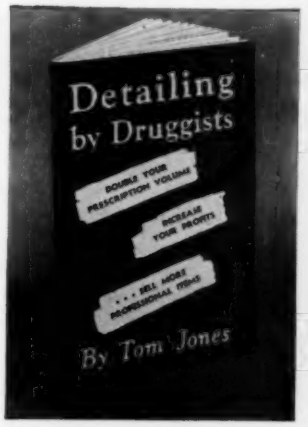
DETAILING BY DRUGGISTS

A Supplement to DETAILING THE PHYSICIAN
and by the same author—TOM JONES

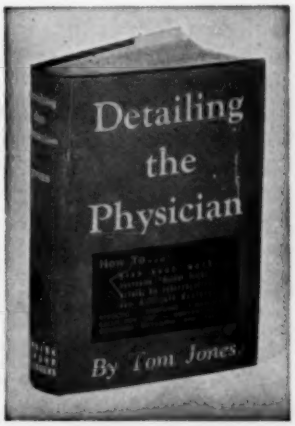
DOUBLE YOUR PRESCRIPTION VOLUME

SELL MORE PROFESSIONAL ITEMS

INCREASE YOUR PROFITS



IF PURCHASED SEPARATELY 75¢ POSTPAID



IF PURCHASED SEPARATELY $2.75 POSTPAID

INCREASE YOUR SALES!

This single volume contains proven methods of productive detailing as practiced by nationally known pharmacists and manufacturers. An indispensable working tool for detail men, sales and advertising managers, pharmacists and surgical dealers.

BOTH BOOKS SENT POSTPAID FOR ONLY $3.00

Send me.....DETAILING BY DRUGGISTS 75¢
.....DETAILING THE PHYSICIAN $2.75
OR BOTH $3.00

Enclosed check for $.....

ROMAINE PIERSON PUBLISHERS, INC.
95 Nassau St. New York, N. Y.

Name.....

Address.....

APPLICATION FOR MEMBERSHIP
IN THE
American Pharmaceutical Association

Approving the objects of the American Pharmaceutical Association, I hereby apply for membership in the Association and subscribe for the "Journal of the American Pharmaceutical Association." I enclose $ for my membership dues and subscription.

Check which you desire:

- ☐ Membership with the PRACTICAL PHARMACY EDITION, at $5.00.
- ☐ Membership with the SCIENTIFIC EDITION, at $6.00.
- ☐ Membership with BOTH EDITIONS, at $7.00.

Name in Full _____
(Print name in full—Initials are not sufficient)

Number and Street _____

Date _____ Town _____ State _____

Paid $_____ No._____

This application with the first year's payment may be sent to the Chairman of the Membership Committee, the Secretary or any officer of the A. Ph. A.

E. F. KELLY, Secretary,
2215 Constitution Ave.,
Washington, D. C.

Assayed FOR APPEAL POTENCY

...*the New* DISPLAYS ON PARKE-DAVIS VITAMINS

Engineered by one of the best display creators in the business, the new Parke-Davis Vitamins Window and Counter Displays were "assayed" by a board of experts. They found these displays highly potent in eye appeal, professional dignity, prestige-building value.

The nine beautiful colors, the third dimensional effects, and the ease with which the new window display can be installed in practically any window—all must be seen to be appreciated.

Ask your Parke-Davis Salesman about the new Vitamins (1) Window Display, (2) Counter Display, (3) Counter Arch Display. Meanwhile keep telling customers about the significance of the PARKE-DAVIS SLOGAN...

The New Counter Display

IF YOU DON'T KNOW YOUR VITAMINS— KNOW THE MAKER!

The New Counter Arch

PARKE, DAVIS & COMPANY • DETROIT, MICHIGAN